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Effect of Oral Administration of Aniline and *p*-Aminodimethylaniline on the Growth of the Rat [1]

By JULIUS WHITE, *senior biochemist*, and JESSE E. EDWARDS, *research fellow, National Cancer Institute, National Institute of Health, United States Public Health Service*

INTRODUCTION

In a previous communication (*1*), evidence was presented which demonstrated that the incorporation of *p*-dimethylaminoazobenzene (butter yellow) into the basal diet of the young rat produced a growth-inhibitory effect (failure to gain weight). The addition of either *l*-cystine or *dl*-methionine to the basal diet still containing the butter yellow resulted in a prompt stimulation in growth. This growth-stimulating effect could not be produced by the addition of amino acid nitrogen as glycine, organic sulfur as taurine, cysteic acid, or inorganic sulfur as sodium sulfate. Recently Kensler, Dexter, and Rhoads (*2*) and Stevenson, Dobriner, and Rhoads (*3*) have isolated, from the urine of rats fed butter yellow, derivatives which indicate that the butter yellow molecule is split by the rat *in vivo* at the azo linkage. It appeared that it would be of interest to study the effect of some of the possible split-products of butter yellow, as indicated by these authors, on the growth of the rat as well as the production of hepatic tumors after oral administration. This report deals with a study of the effect of the oral administration of two possible breakdown products of butter yellow, aniline and *p*-aminodimethylaniline, on the growth of the rat.

[1] *Presented before the 29th annual meeting of the American Society of Biological Chemists, Boston, March 31–April 4, 1942.*

EXPERIMENTAL PROCEDURE AND RESULTS

Male Osborne-Mendel rats at weaning were housed in individual cages and placed on a diet of the following composition:

	Percent
Casein	6
Corn starch	50
Crisco	24
Sucrose	15
Cod-liver oil	1
Inorganic salts [1]	4
	100

[1] Modified Osborne-Mendel salt mixture (*4*).

In addition each rat received a daily supplement of 400 mg. of dried brewers' yeast and one drop of cod-liver oil. The same procedure as previously described (*1*) was employed here. The animals were allowed to ingest the basal diet until they

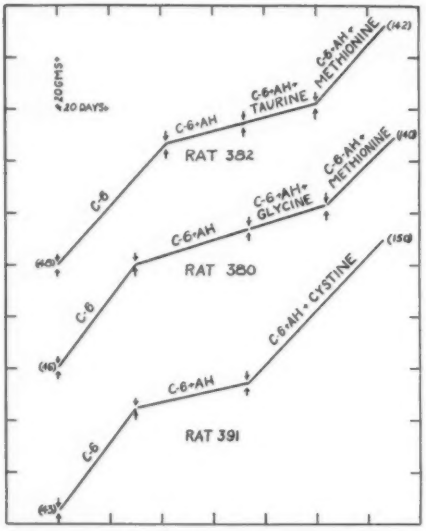

FIGURE 1.—Growth of rats on the basal diet and on basal diet with added aniline hydrochloride, as influenced by various supplements. The diet used in a portion of an experiment is indicated between two sets of arrows representing the beginning and end of a period. The symbols used in this figure are explained in table 1. The initial and final body weights are shown in parentheses for animals Nos. 382, 380, and 391. These are representative of 25, 19, and 35 rats, respectively.

had reached a weight of 75 to 80 gm. At this point either aniline hydrochloride (80 mg.) or *p*-aminodimethylaniline monohydrochloride (120 mg.) was incorporated into each 100 gm. of the basal diet. The hydrochlorides were used in preference to the free bases since the latter tend to oxidize very rapidly on exposure to the air. The amount of each amine finally used in this study was chosen from a number of preliminary experiments which had demonstrated that these dosages would markedly inhibit the weight increment of the rat for long periods of time without any gross evidence of toxicity in the animal. After the growth-retarding effect was demonstrated for a suitable period, the addition of *l*-cystine (500 mg.) or *dl*-methionine (500 mg.) to each 100 gm. of basal diet still containing the amine resulted in a prompt stimulation in growth which approximated the weight increase observed in rats ingesting the basal diet alone. However, the addition of glycine, taurine, or cysteic acid failed to stimulate growth. These data are in accord with those reported previously for growth retardation by butter yellow (*1*).

The data (table 1 and figs. 1–2) indicate that the oral administration of either aniline hydrochloride or *p*-aminodimethylaniline hydrochloride to the young rat behaves in a manner similar to that reported for bromobenzene (*5*), naphthalene (*6*), anthracene (*7*), iodoacetic acid (*8*), methylcholanthrene, benzpyrene, or pyrene (*9*), and *p*-dimethylaminoazoben-

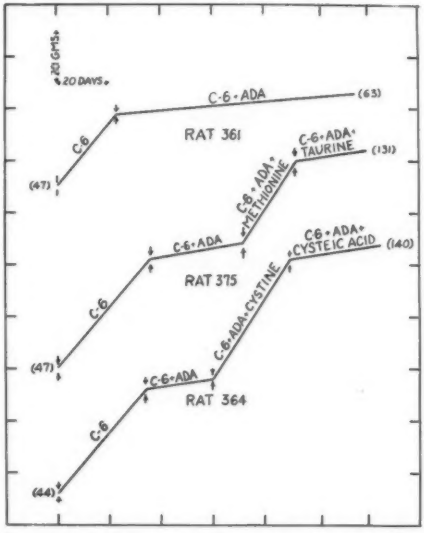

FIGURE 2.—Growth of rats on basal diet and on basal diet with added *p*-aminodimethylaniline, as influenced by various supplements. Designations for body weights and diets employed are the same as in figure 1. Animals Nos. 361, 375, and 364 are typical for these experiments and represent 32, 30, and 31 animals, respectively.

zene (*1*). As far as we know, these experiments demonstrate for the first time that the oral administration of aromatic amines represented by aniline and *p*-aminodimethylaniline are capable of retarding the growth of the young rat (failure to gain weight).

TABLE 1.—*Summary of average daily weight changes and average daily food consumption of animals receiving basal diets and various types of supplements.*

Diet	Description of diet in figures 1-2	Number of animals	Average daily weight change	Average daily food consumption
			Gms.	*Gms.*
Basal	C-6	172	+1.7	7.6
Basal + aniline hydrochloride	C-6+AH	79	+.2	6.1
Basal + aniline hydrochloride + taurine		25	+.3	6.3
Basal + aniline hydrochloride + methionine		25	+1.5	7.9
Basal + aniline hydrochloride + glycine		19	+.3	6.6
Basal + aniline hydrochloride + cystine		35	+1.5	7.7
Basal + *p*-aminodimethylaniline hydrochloride	C-6+ADA	93	+.2	6.0
Basal + *p*-aminodimethylaniline hydrochloride + methionine		30	+1.4	7.7
Basal + *p*-aminodimethylaniline hydrochloride + taurine		30	+.3	6.4
Basal + *p*-aminodimethylaniline hydrochloride + cystine		31	+1.4	6.1
Basal + *p*-aminodimethylaniline hydrochloride + cysteic acid		31	+.3	6.1

SUMMARY

The addition of aniline hydrochloride or *p*-aminodimethylaniline to a basal diet of relatively low protein content produced a retardation of growth (failure to gain weight normally) when administered to young rats.

This retardation in growth can be overcome by adding *l*-cystine or *dl*-methionine to the diet still containing either of the amines, and growth results can be obtained which are comparable with those observed in animals ingesting the basal diet alone.

The addition of an oxidized product of cystine, cysteic acid, or of the decarboxylated product of cysteic acid, taurine, failed to stimulate growth. This is meant to indicate that sulfur in the form of cystine, methionine, or substances giving rise to cystine or methionine are capable of functioning as growth restorers in animals ingesting aniline or *p*-aminodimethylaniline.

REFERENCES

(*1*) WHITE, J.: Retardation of growth of the rat ingesting *p*-dimethylaminoazobenzene (butter yellow). I. The effect of various dietary supplements. J. Nat. Cancer Inst., 1: 337–341 (1940.)

(*2*) KENSLER, C. J., DEXTER, S. O., and RHOADS, C. P.: The inhibition of a diphosphorpyridine nucleotide system by split products of dimethylaminoazobenzene. Cancer Research, 2: 1–10 (1942).

(*3*) STEVENSON, E. S., DOBRINER, K., and RHOADS, C. P.: The metabolism of dimethylaminoazobenzene (butter yellow) in rats. Cancer Research, 2: 160–167 (1942)

(*4*) HUBBELL, R. B., MENDEL, L. B., and WAKEMAN, A. J.: New salt mixture for use in experimental diets. J. Nutrition, 14: 273–285 (1937).

(*5*) WHITE, A., and JACKSON, R. W.: The effect of bromobenzene on the utilization of cystine and methionine by the growing rat. J. Biol. Chem., 111: 507–513 (1935).

(*6*) STEKOL, J. A.: Studies on the mercapturic acid synthesis in animals. V. The effect of naphthalene on the growth of rats as related to diets of varying sulfur content. J. Biol. Chem., 121: 87–91 (1937).

(*7*) BOYLAND, E., and LEVI, A. A.: Metabolism of polycyclic compounds. III. Anthrylmercapturic acid. Biochem. J., 30: 1225–1227 (1936).

(*8*) STEVENSON, E. S., and WHITE, A.: Influence of iodoacetic acid on sulfur metabolism. Growth studies in the young rat. J. Biol. Chem., 134: 709–720 (1940).

(*9*) WHITE, J., and WHITE, A.: Inhibition of growth of the rat by the oral administration of methylcholanthrene, benzpyrene, or pyrene, and the effects of various dietary supplements. J. Biol. Chem., 131: 149–161 (1939).

Effect of Dietary Cystine on the Development of Hepatic Tumors in Rats Fed *p*-Dimethylaminoazobenzene (Butter Yellow)[1]

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INTRODUCTION

The classical observations of Sasaki and Yoshida (*1*) and Kinosita (*2–3*) in producing hepatic tumors in rats by the oral administration of *o*-aminoazotoluene and *p*-dimethylaminoazobenzene, respectively, have opened a whole new field in cancer research. Indeed, a new tool has been made available for the study of dietary influences on carcinogenesis.

With few exceptions the basal diet used by investigators since the first report of hepatic tumor development in the rat has been rice supplemented with a small piece of carrot. Thus often in reported experiments on the results of dietary studies, rice was used as the basal constituent to which were added the various supplements under specific investigation.

Sugiura and Rhoads (*4*) observed that hepatic tumors occurring in rats maintained on a diet of unpolished rice and butter yellow could be prevented for a period of 150 days by the addition of rice-bran extract. They also observed that the addition of 15 percent brewers' yeast prevented hepatic tumors for periods as long as 284 days. Kensler, Sugiura, Young, Halter, and Rhoads (*5*) reported that the addition of riboflavin and casein to a diet of rice and butter yellow greatly retarded the incidence of hepatic tumors. Mori (*6*) showed that the addition of cystine to a hepatic-cancer-producing diet of rice and butter yellow failed to alter the incidence of tumors. Other reports dealt with attempts to alter the incidence of hepatic tumors induced by butter yellow in animals on this basal rice diet by the addition of various supplements.

Few papers have appeared in which hepatic-tumor formation has been studied on diets other than rice. György, Poling, and Goldblatt (*7*) showed that rats ingesting a low protein diet (6 percent casein) containing butter yellow would develop adenocarcinomas and malignant hepatomas. The addition of both cystine and choline afforded "a definite but not regular protection against pathological changes in the liver" in a period of 175 days. Miller, Miner, Rusch, and Baumann (*8*) made an extensive study of the effect of various dietary constituents on the production of hepatic tumors by the oral administration of butter yellow. These authors found that high levels of dietary casein, as well as liver, yeast, or egg, afforded protection for 4–6 months against the production of liver tumors in rats that were fed butter yellow only 4 months. If the dye was fed continuously in a diet containing liver, tumors developed eventually. In a study of 34 different diets, these authors concluded that in general—

(a) the nutritionally adequate diets offered at least partial protection against hepatoma formation; (b) the protective supplements were usually

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rich in both protein and vitamin B complex, particularly riboflavin; (c) the nonprotective diets were deficient in at least one of these factors.

It was previously reported (*9*) that the addition of butter yellow to a basal diet of relatively low protein content produced a retardation of growth when administered to young rats. Furthermore, the addition of either *l*-cystine or *dl*-methionine to the basal diet still containing the butter yellow resulted in a prompt stimulation in growth.

It occurred to us that if sulfur-containing amino acids participated in the detoxication of orally administered butter yellow, a retarding influence might be obtained in the development of hepatic tumors when rats were fed a butter-yellow diet with restricted cystine. This report deals with the results obtained by the continuous oral administration of butter yellow to rats ingesting high and low cystine diets, respectively. The pathologic changes and classification of the hepatic tumors which arose during the course of this study have already been reported (*10*).

EXPERIMENTAL PROCEDURE AND RESULTS

Both male and female Osborne-Mendel rats at weaning (21-28 days) were placed in individual cages where they were allowed to eat ad libitum a basal diet of the following composition:

	Percent
Casein [1]	6
Corn starch	50
Crisco	24
Sucrose	15
Cod-liver oil	1
Inorganic salts [2]	4
	100

[1] Furnished by the Casein Corporation of America.
[2] Modified Osborne-Mendel salt mixture (*11*).

In addition each animal received a daily supplement of 400 mg. of dried brewers' yeast.[2] When the animals had attained a weight of 75-86 gm., 60 mg. of butter yellow was added to each 100 gm. of the basal diet. The weight of each animal, as well as the food consumed, was determined twice weekly throughout the course of the experiment. The results were based on 167 animals studied pathologically from the one hundred and twentieth day after the experiment was started. Eighty-seven of these rats were given a supplement of 0.5 percent *l*-cystine at the time when the butter-yellow diet was started. This is referred to as the high cystine-butter yellow diet, or cystine-supplemented diet. The remaining 80 animals had no additional supplement, and the diet for this group is referred to as the low cystine-butter yellow diet. Occasionally an animal would scatter food, but no difficulty was encountered in accounting for the loss since the scattered food was collected on a sheet of paper placed below the raised screen floor of the cage.

The butter yellow produced no apparent toxic effects since only four animals died during the course of the experiment, two from pneumonia at 4 months and two because of hair balls in the stomach at 7 months. This marked low mortality is in striking contrast with that of animals ingesting rice as the basal diet. The Japanese investigators reported a 40-percent mortality in the first month of their experiment. Miller, Miner, Rusch, and Baumann (*8*) also reported a high mortality. Sugiura and Rhoads (*9*) observed that the rats on a similar basal-butter yellow diet supplemented with a small slice of fresh carrot daily survived longer. All the animals ingesting the high cystine-butter yellow diet showed a continuous gain in weight averaging 0.71 gm. per day, while the animals ingesting the low cystine-butter yellow diet showed an average daily gain of 0.3 gm.

[2] *Harris dried brewers' yeast.*

Two groups of rats ingesting the high and the low cystine diet, respectively, with no butter-yellow supplement, were used as controls. Animals ingesting the high cystine diet gained an average of 1.6 gm. per day, while those on the low cystine diet gained an average of 0.6 gm. per day.

Most of the animals on the butter-yellow diets were killed from 14 to 392 days after the beginning of the experiment, the majority between the one hundred and fortieth and the two-hundredth day, and a few as late as 500 days. Inasmuch as no tumor was found either grossly or microscopically in either experimental group before 120 days, the data are based on 167 animals ingesting the diets for periods between 120 and 500 days.

The tumor masses in the livers of the animals could be palpated through the abdominal wall. Invariably these masses became greatly enlarged within a week after they were first recognized. The rats were killed periodically and autopsied, and a microscopic examination was made on the liver as well as other tissues. Lesions considered as primary carcinoma of the liver were so diagnosed on the basis of the microscopic examination.

Eighty-seven rats on the high cystine diet were thus palpated and killed: 35 of the 87 were killed between 120 and 150 days after the beginning of the experiment, and of the 35 only 3 were negative for hepatic tumor; 47 of the rest were killed in the period between 150 and 200 days, and all were found to have malignant tumors of the liver; the 5 remaining were killed in the period between 200 and 500 days, and all showed hepatic tumors.

In the low cystine-butter yellow group the animals were killed at periods corresponding to those for the high cystine-butter yellow group. In the period between 120 and 180 days, 13 rats in this group were killed and autopsied, and 2 showed hepatic carcinomas; between 180 and 200 days an additional 13 were killed, and 2 of them were positive for hepatic carcinoma; between 200 and 390 days, 31 animals showed pathologic evidence of primary cancer of the liver; of the remaining 23 animals, 13 developed tumors by 500 days, and 10 had none.

The data on hepatoma formation in both groups are shown graphically in figure 1. At the end of 200 days, 79 (96.3 percent) of the 82 autopsied animals on the high cystine-butter yellow diet had primary carcinomas, as compared with 4 (15.4 percent) of the 26 on the low cystine-butter yellow diet. At a glance these figures are very striking; and if the experiment had been terminated at this point, one could report a marked reduction in tumor formation among animals on the low cystine diet. However, a continuation of the experiment indicated that the major effect is one of increased latent period rather than one of prevention of tumor formation. It is conceivable that the difference between 96.5 and 61.4 percent tumor formation at the end of 390 days in the two groups may be significant since the cystine content of the low cystine diet was only relatively low, i. e., the level of cystine had not been reduced to the point at which

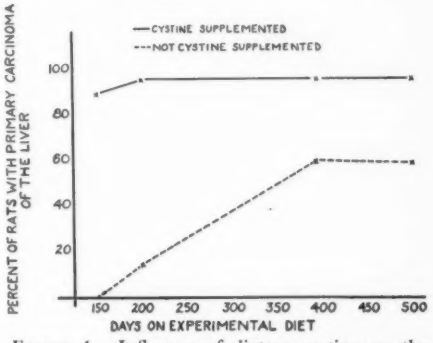

FIGURE 1.—Influence of dietary cystine on the incidence of cancer of the liver in the rat induced by butter yellow.

growth had been almost completely retarded. Experiments are now in progress to determine whether a markedly low cystine diet will increase the latent period still further or possibly prevent hepatic tumor formation.

SUMMARY

The production of hepatic tumors in rats by the oral administration of butter yellow in diets of relatively high and low cystine content is reported.

At the end of 200 days, 79 (96.3 percent) of the 82 animals ingesting the high cystine-butter yellow diet that were autopsied, developed primary hepatic carcinoma, while only 4 (15.4 percent) of the 26 animals on the low cystine-butter yellow diet that were autopsied, developed such tumors. At 390 days, a total of 35 of the 57 animals, or 61.4 percent of the low cystine group, developed hepatomas; and at 500 days, 48, or 60.0 percent of this group, developed cancer of the liver.

The major effect of the low cystine diet appears to be one of increasing the latent period rather than the prevention of tumor formation.

REFERENCES

(*1*) Sasaki, T., and Yoshida, T.: Experimentelle Erzeugung des Lebercarcinoms durch Fütterung mit o-Amidoazotoluol. Virchows Arch. f. path. Anat., 295: 175–200 (1935).

(*2*) Kinosita, R.: Special report. Studies on the carcinogenic chemical substances. Tr. Soc. Path. Jap., 27: 665–727 (1937).

(*3*) ———: Studies on the cancerogenic azo and related compounds. Yale J. Biol. & Med., 12: 287–300 (1940).

(*4*) Sugiura, K., and Rhoads, C. P.: Experimental liver cancer in rats and its inhibition by rice-bran extract, yeast, and yeast extract. Cancer Research, 1: 3–16 (1941).

(*5*) Kensler, C. J., Sugiura, K., Young, N. F., Halter, C. R., and Rhoads, C. P.: Partial protection of rats by riboflavin with casein against liver cancer caused by dimethylaminoazobenzene. Science, 93: 308–310 (1941).

(*6*) Mori, K.: On the effect of cystine feeding on experimental production of liver cancer. Gann, 35: 121–125 (1941).

(*7*) György, P., Poling, E. C., and Goldblatt, H.: Necrosis, cirrhosis and cancer of liver in rats fed a diet containing dimethylaminoazobenzene. Proc. Soc. Exper. Biol. & Med., 47: 41–44 (1941).

(*8*) Miller, J. A., Miner, D. L., Rusch, H. P., and Baumann, C. A.: Diet and hepatic tumor formation. Cancer Research, 1: 699–708 (1941).

(*9*) White, J.: Retardation of growth of the rat ingesting *p*-dimethylaminoazobenzene (butter yellow). I. The effect of various dietary supplements. J. Nat. Cancer Inst., 1: 337–341 (1941).

(*10*) Edwards, J. E., and White, J.: Pathological changes, with special reference to pigmentation and classification of hepatic tumors in rats fed *p*-dimethylaminoazobenzene (butter yellow). J. Nat. Cancer Inst., 2: 157–183 (1941).

(*11*) Hubbell, R. B., Mendel, L. B., and Wakeman, A. J.: New salt mixture for use in experimetal diets. J. Nutrition, 14: 273–285 (1937).

Pathology of Rat Hepatoma 31

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The tumor described in this paper, a transplantable induced hepatoma in the rat, has been studied rather extensively from the chemical and metabolic points of view by members of the National Cancer Institute staff (*1–8*). Since it is still an object of investigation and its continued use for some time to come is anticipated, it is considered pertinent to record its history and pathologic characteristics. The tumor is designated as rat hepatoma 31.

HISTORY OF TUMOR

The primary tumor arose in the liver of a male Osborne-Mendel rat. At the age of 1 month the animal had been placed on the basal diet of White and Jackson (*9*) supplemented continuously with 0.06 percent *p*-dimethylaminoazobenzene (butter yellow) and intermittently with 0.5 percent cystine and 0.5 percent methionine.

At the end of 6 months on this regimen, on May 7, 1940, the animal died; and autopsy was performed shortly thereafter while the body was still warm. At this time pieces of tumor tissue were transplanted by the trocar technique into the subcutaneous tissue of several rats of the homologous strain. The tumor, now in the sixteenth generation, has continued to grow satisfactorily in about 80 percent of the animals inoculated, reaching an average diameter of 2.0 cm. in 30 days.

GROSS PATHOLOGY

Primary Tumor

At autopsy the animal bearing the primary hepatic tumor weighed 205 gm. The liver was greatly enlarged, weighing 68 gm. All the major lobes were irregular in contour because of innumerable pale gray to purple tumor masses measuring 0.5 to 2.0 cm. in diameter which had obliterated or obscured much of the hepatic tissue. The minor lobes showed only a few small tumor nodules and otherwise were firm, yellowish-brown, and finely granular. No other tumor tissue was observed in the body.

Transplants

In the subcutaneous tissues the transplants were discrete, ovoid, or somewhat lobulated, firm to hard, pale gray masses. The cut surface of small transplants was homogeneous and pearly gray, while in those nodules larger than 1.0 cm. in diameter there was a peripheral, firm, pearly gray, glistening zone about 4.0 mm. thick and a central zone of softer, friable, dull, yellowish-gray tissue.

No visceral metastases were observed, but in one animal carrying a third-generation transplant, as will be indicated below, there was microscopic evidence of metastasis to a pretrachial superior mediastinal lymph node.

MICROSCOPIC PATHOLOGY

Primary Tumor

Sections taken through representative areas of the liver revealed the two classical types of induced hepatic tumors of the rat, hepatoma and adenocarcinoma. The tumor growing as hepatoma could further be divided into two subgroups, hepatoma type I and hepatoma type II, described in an earlier paper (*10*).

The tissue growing as hepatoma type I was composed of cords of tumor cells resembling hepatic parenchymal cells very closely, with acidophilic cytoplasm, well-defined cytoplasmic membranes, and relatively large vesicular nuclei each containing one to three prominent basophilic nucleoli. Such epithelial cords alternated with wide, and at times cavernous, endothelial-lined, blood-filled sinuses (fig. 1, *A*). There were a few wide cysts lined by flattened tumor cells.

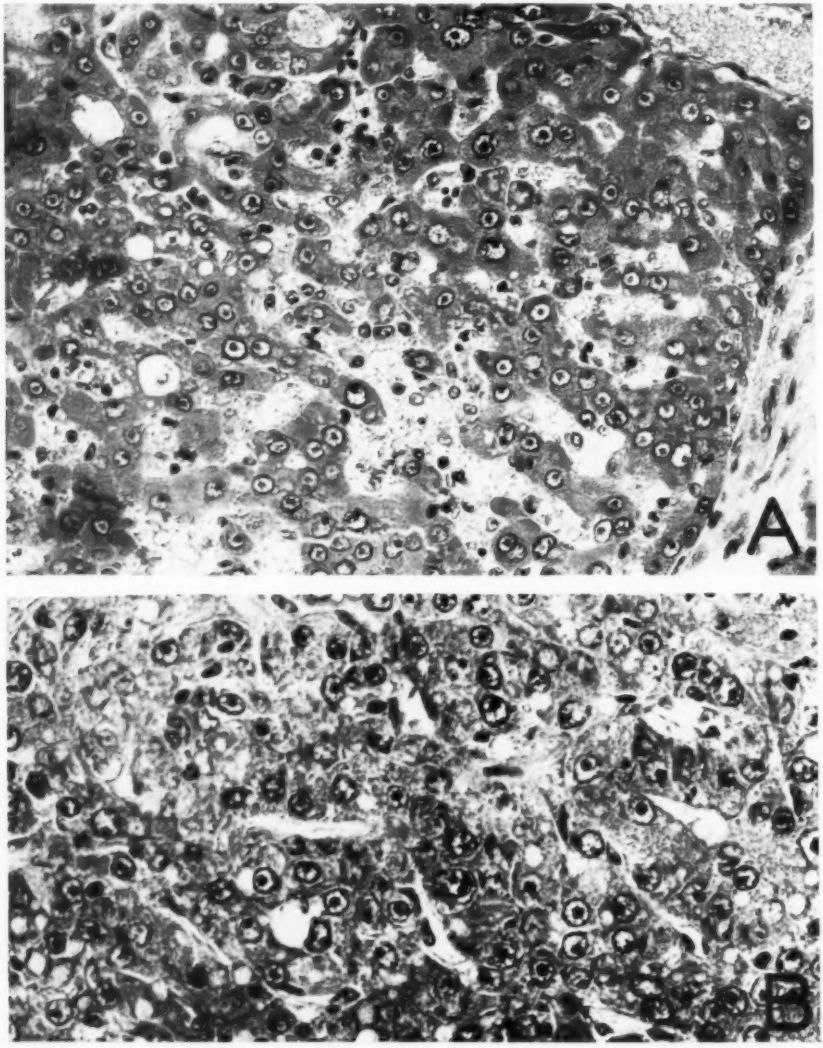

FIGURE 1.—Primary tumor. Haematoxylin and eosin. *A*, Hepatoma type I. × 320; *B*, Hepatoma type II with solid cords. × 430.

In addition to the hepatoma type I, there were areas in which the neoplasm took the form of hepatoma type II. Such tissue was characterized by a structure, fundamentally hepatic, in which epithelial cords alternated with endothelial-lined sinuses, in this respect resembling hepatoma type I. The outstanding differences were reflected in the individual tumor cells which here showed faintly basophilic, at times vacuolated, cytoplasm and ill-defined cytoplasmic membranes, the latter being responsible for an apparently syncytial character to the cords (fig. 1, *B*). Although many of the epithelial cords were solid, some showed acinar formations lined by cells identical in appearance with those of the solid cords. Such acini were either independent structures or were anatomically continuous with solid areas. In either case they maintained a characteristic relationship to endothelial-lined blood sinuses (fig. 2, *A*).

In the areas diagnosed as adenocarcinoma the tumor cells formed varying sized spaces which were supported by an abundance of connective tissue and lacked the characteristic relationship to the blood vessels exhibited by the epithelial cells in hepatoma (fig. 2, *B*.) The spaces formed by the neoplastic cells varied in appearance, some being relatively narrow and empty, others wider. Some of the latter were filled with mucus which compressed and flattened the lining cells. There were also cysts with papillary structures projecting into the lumens.

Transplants

A striking regularity existed in the appearance of the tumor cells in all the transplants although the architecture of the tumor was not identical in all generations. The tumor cells were large with visible cytoplasmic membranes and heavily staining eosinophilic and strongly osmiophilic cytoplasm. The nuclei were relatively large, vesicular, and reniform or lobulated. The nuclear membrane was thick, and each nucleus contained one to three large prominent basophilic nucleoli. Mitotic figures, usually typical, were numerous, and there were rare multinucleated and uninucleated giant cells.

No cytoplasmic pigment, inclusions, vacuoles, fibrils, or intercellular bridges were present. Glycogen as tested with Best's carmine stain was absent even after forced feeding of glucose. Fat, as demonstrated by the sudan IV stain, was usually absent, only small droplets being present in the vicinity of necrotic areas. The polarity of the cells was disturbed, resulting in a haphazard arrangement of the nuclei in relation to the free borders of the cells.

Both the mitochondria [1] and the Golgi apparatus [2] of tumor cells showed characteristic differences from the normal. The mitochondria of normal liver cells of the Osborne-Mendel rat show the typical pattern as described for the Wistar strain white rat by Steffens (*11*). Mitochondria of cells at the periphery of the lobule appear regularly as plump rods and spheres (fig. 3, *A*), while those at the margin of the central vein are numerous, long, slender, lightly staining filaments (fig. 3, *B*). The tumor cells differed from normal hepatic cells by possessing relatively few, small, filamentous mitochondria (fig. 3, *C*). Rarely, viable tumor cells near areas of necrosis were found to contain spherical mitochondria. With this exception, no other variation from the usual mitochondrial form of the tumor cells was encountered. The mitochondria of the tumor cells differed not only from those

[1] *Mitochondrial preparations were made by a modified Regaud method: fixation in cold Regaud's fluid for 3 days followed by post-chromation in 5 percent potassium bichromate at 37° C. for 3 days.*

[2] *The Golgi apparatus was demonstrated by the Kolatchew-Nassanow method with 4 to 5 days' post-osmication.*

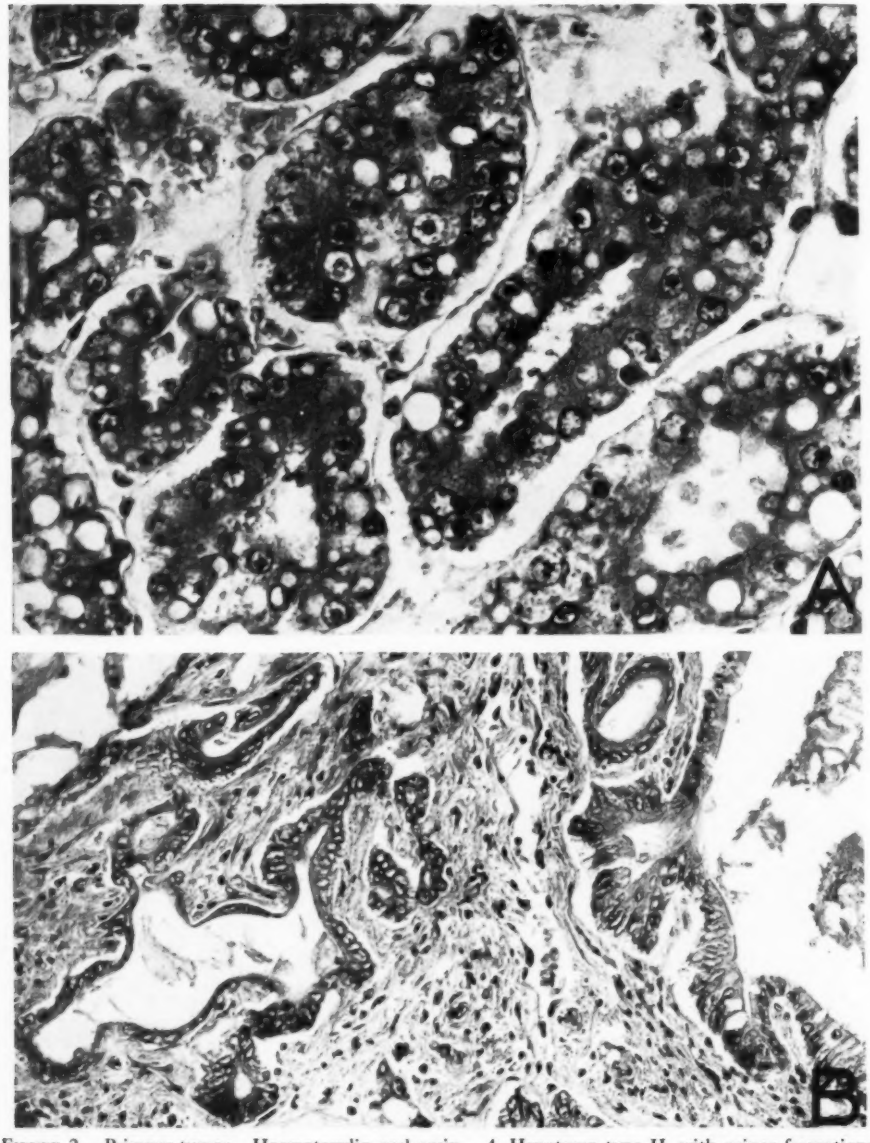

FIGURE 2.—Primary tumor. Haematoxylin and eosin. *A*, Hepatoma type II, with acinar formations. × 500; *B*, Adenocarcinoma. × 280.

of normal hepatic cells but also from those of normal bile duct epithelium in which the mitochondria are relatively few in number but are long slender filaments.

The main portion of the Golgi apparatus of the normal hepatic parenchymal cell is

located close to that portion of the cell membrane forming part of the bile canaliculus (fig. 4, *A*). In the tumor cells the Golgi apparatus existed as a greatly expanded, open network situated on one side of the cell between the nucleus and the cell membrane. In areas showing an acinar arrangement of the cells the Golgi

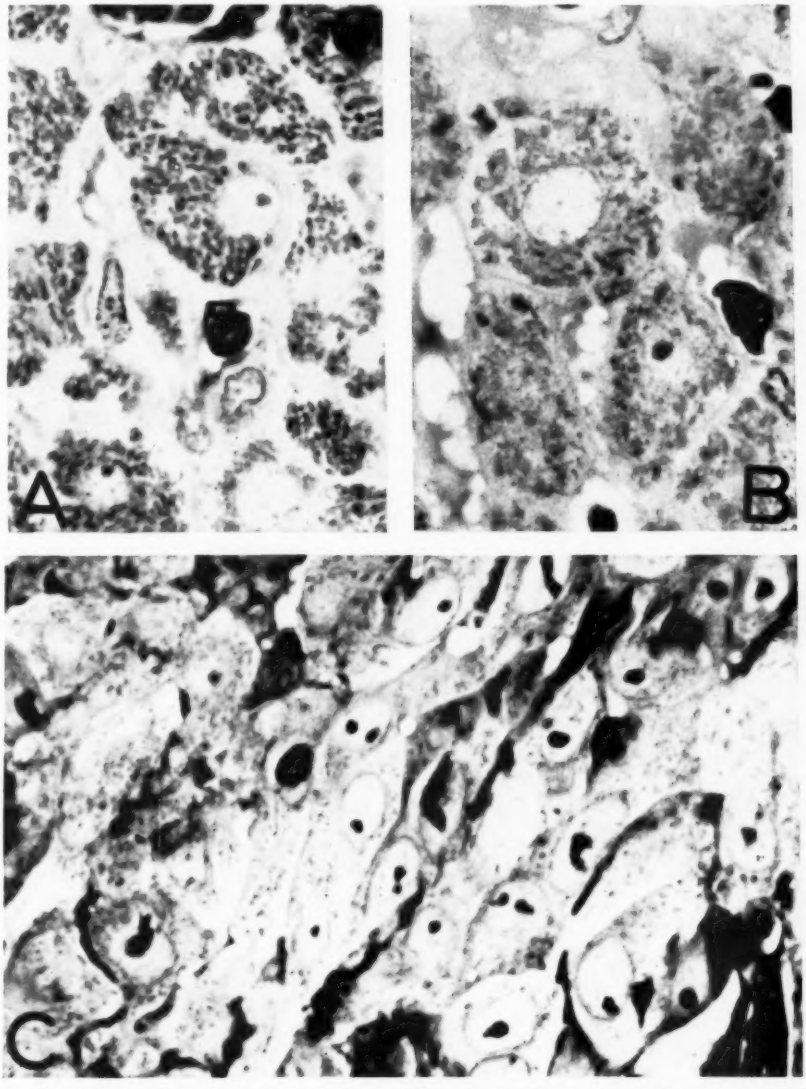

FIGURE 3.—Mitochondria. Regaud method. × 1300; *A*, Periphery of normal rat hepatic lobule, mitochondria plump rods and spheres; *B*, Center of normal rat hepatic lobule, mitochondria long slender filaments; *C*, Transplant of hepatoma 31, mitochondria few small filaments.

material was always found between the nucleus and the free surface of the cell. In thin sections the apparatus appeared as a group of dispersed osmiophilic filaments rather than as an anastomosing network (fig. 4, *B*). A second type of Golgi apparatus was occasionally found. It occurred in smaller tumor cells as a small, compact knot of osmiophilic material located close to the nuclear membrane. Since these small cells occurred in pairs or groups and since intermediate types of Golgi apparatus between the condensed and expanded forms were occasionally found, it was inferred that the former had recently undergone mitosis.

The Golgi apparatus of the tumor cells was also readily distinguished from that of normal bile duct epithelium. In bile duct cells the main portion of the Golgi material is found in close contact with the nuclear membrane on the side toward the free surface of the cell, but in addition it extends down around the lateral borders of the nucleus as single, smooth, heavy strands.

The arrangements of the neoplastic cells in the first two generations of the transplants were similar. There were relatively few two-cell-thick cords and very many single-layered, spherical or elongated, branched, narrow acini (fig. 5, *A*). The relationship between the epithelium and the stroma was identical with that exhibited by hepatic tissue. Immediately surrounding each epithelial cord or acinus was a layer of relatively thick reticulum which separated the epithelium from a narrow endothelial-lined sinus (fig. 5, *B*).

In the third generation the tumor resembled in part the first- and second-generation transplants, while other areas exhibited a structure of a predominantly solid nature, a picture identical with that in all the later generations. The usual arrangement was that of numerous closely packed cells (fig. 6, *A*), in some cases set off into obvious cords surrounded by heavy reticulum. In some of the areas the cells of the sheets were in actual contact with each other. However, apparent sheets of epithelial cells frequently showed subdivision by numerous relatively delicate strands of reticulum into cords, often parallel, and varying from one to about six cells in thickness (fig. 7, *A* and *B*). Occasionally, there were a few isolated acini mingled with the solid sheets (fig. 6, *B*).

Another arrangement was encountered on a few occasions. This was characterized by relatively narrow branching cords of tumor cells lying in a framework of abundant connective tissue with considerable collagen and numerous fibroblasts (fig. 8, *A*). In one such area in a thirteenth-generation transplant, there was membranous bone formation (fig. 8, *B*). No bone was observed in subsequent transplant generations.

Tissue of a fifteenth-generation transplant was tested microchemically for alkaline phosphatase according to the method of Gomori (*12*). With this method, sodium glycerophosphate is used as the substrate. The tumor showed an abundance of alkaline phosphatase in concentration comparable with that shown by the intestinal mucosa and the renal cortex of the animal bearing the tumor. There was a far greater concentration in the tumor than in the liver of the same rat. The distribution of the enzyme was interesting, the enzyme being present in relatively small amounts within the viable tumor cells and in great abundance in the necrotic zones.

A moderate amount of calcium occurring spontaneously was observed in the necrotic zones of duplicate sections stained with Gomori's method save for the absence of glycerophosphate. This reaction was considerably overshadowed in those sections tested for phosphatase activity with the substrate containing sodium glycerophosphate (figs. 9 and 10).

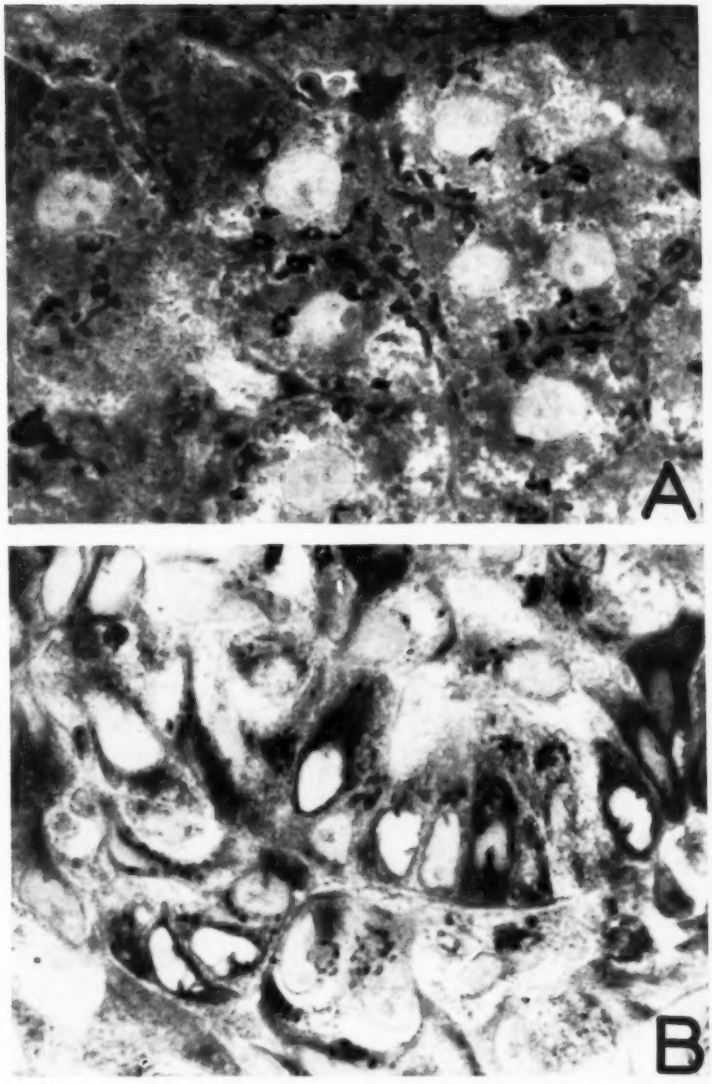

FIGURE 4.—Golgi apparatus, Kolatchew-Nassonow method. × 1300. *A*, Normal rat liver, Golgi apparatus showing largely peripheral distribution along margin of cell forming part of bile canaliculus; *B*, Transplant of hepatoma 31, Golgi apparatus forming coarse network in a juxtanuclear position.

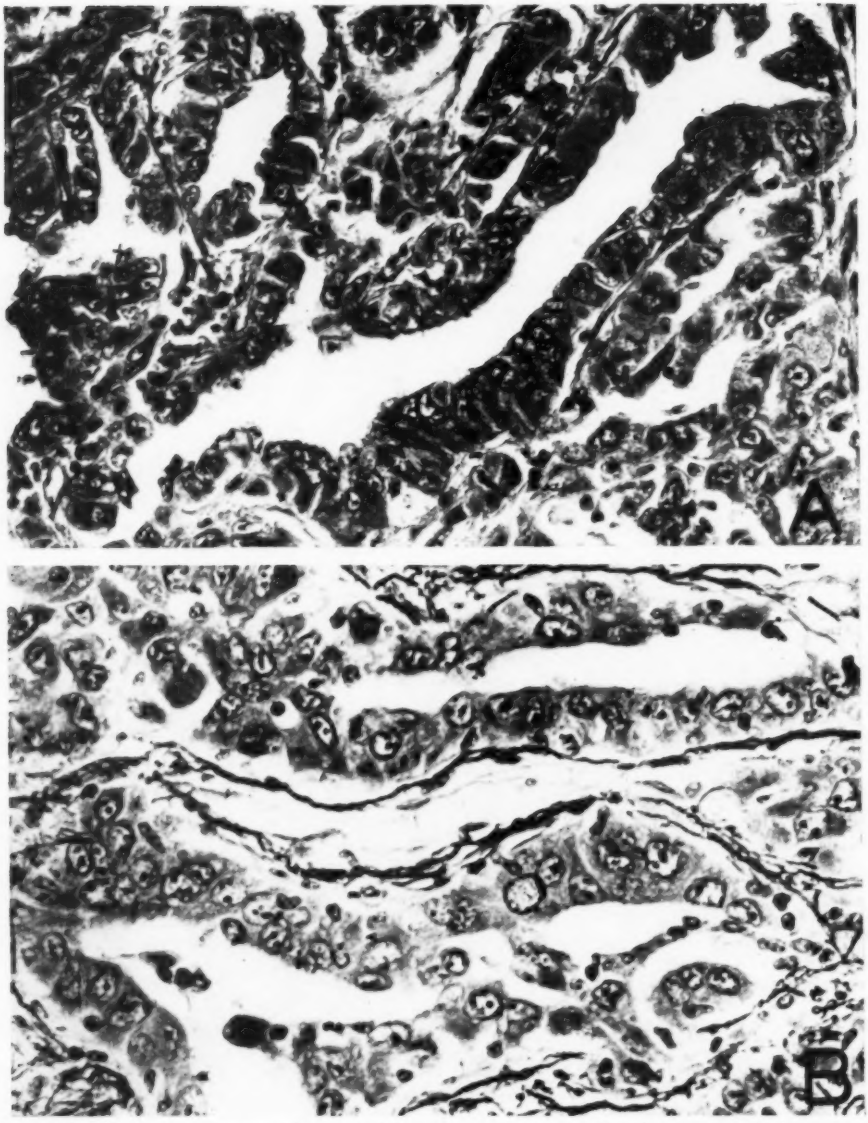

FIGURE 5.—First-generation transplant. *A*, Adenomatous structure. Phosphotungstic acid haematoxylin. × 400; *B*, Liverlike relationship between epithelium and blood capillary. Laidlaw's reticulum stain. × 490.

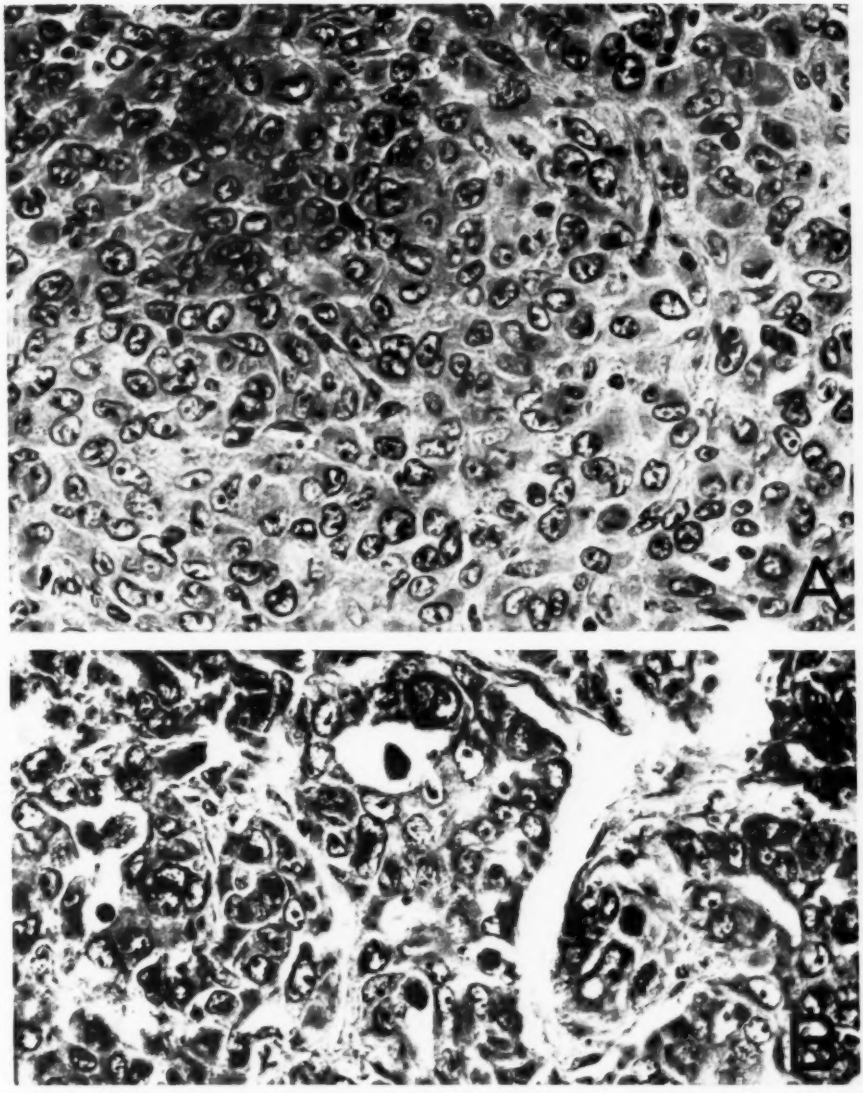

FIGURE 6.—*A*, Solid tumor in eleventh-generation transplant. Haematoxylin and eosin. × 465; *B*, Occasional adenomatous structures seen in tumor predominately solid. Third generation. Phosphotungstic acid haematoxylin. × 400.

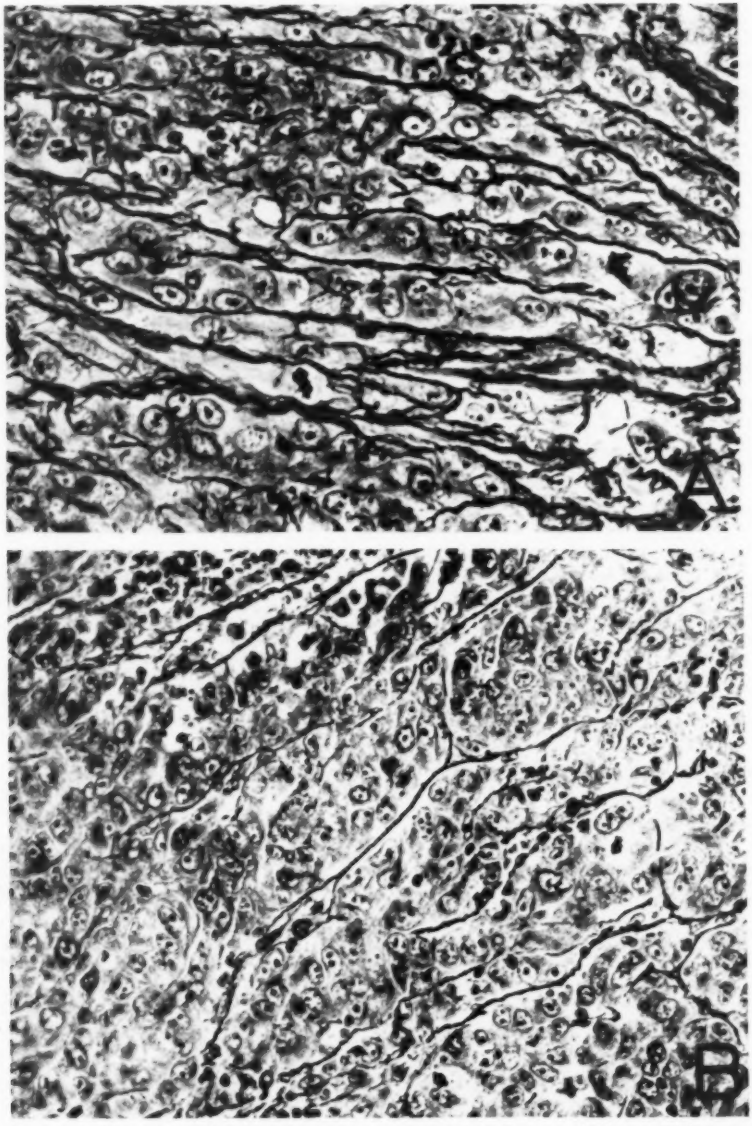

FIGURE 7.—*A*, Solid tumor subdivided into narrow parallel cords by reticulum. Eighth generation. × 495; *B*, Wider cords of tumor cells. Thirteenth generation. × 355. Laidlaw's stain for reticulum used in both *A* and *B*.

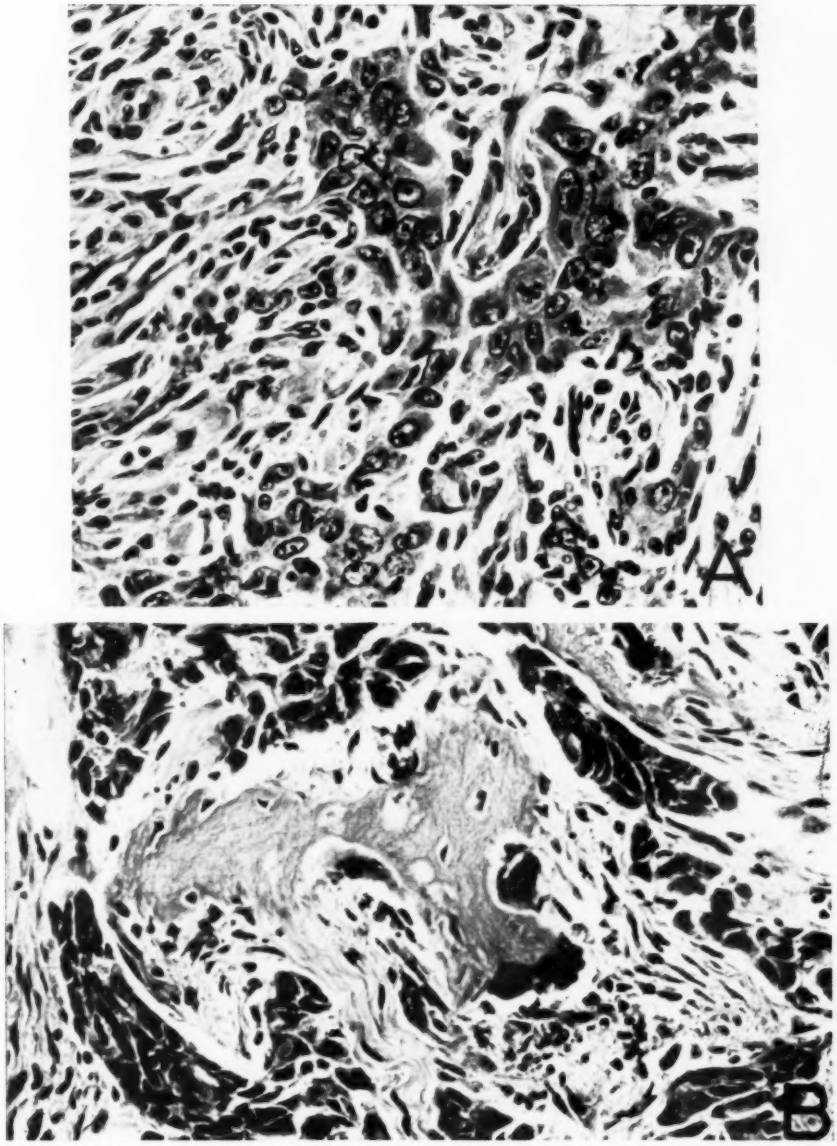

FIGURE 8.—*A*, Narrow, branched cords of tumor cells lying in dense cellular connective tissue. Tenth generation. Haematoxylin and eosin. × 500; *B*, Membranous bone in stroma of a thirteenth-generation transplant. Haematoxylin and eosin. × 390.

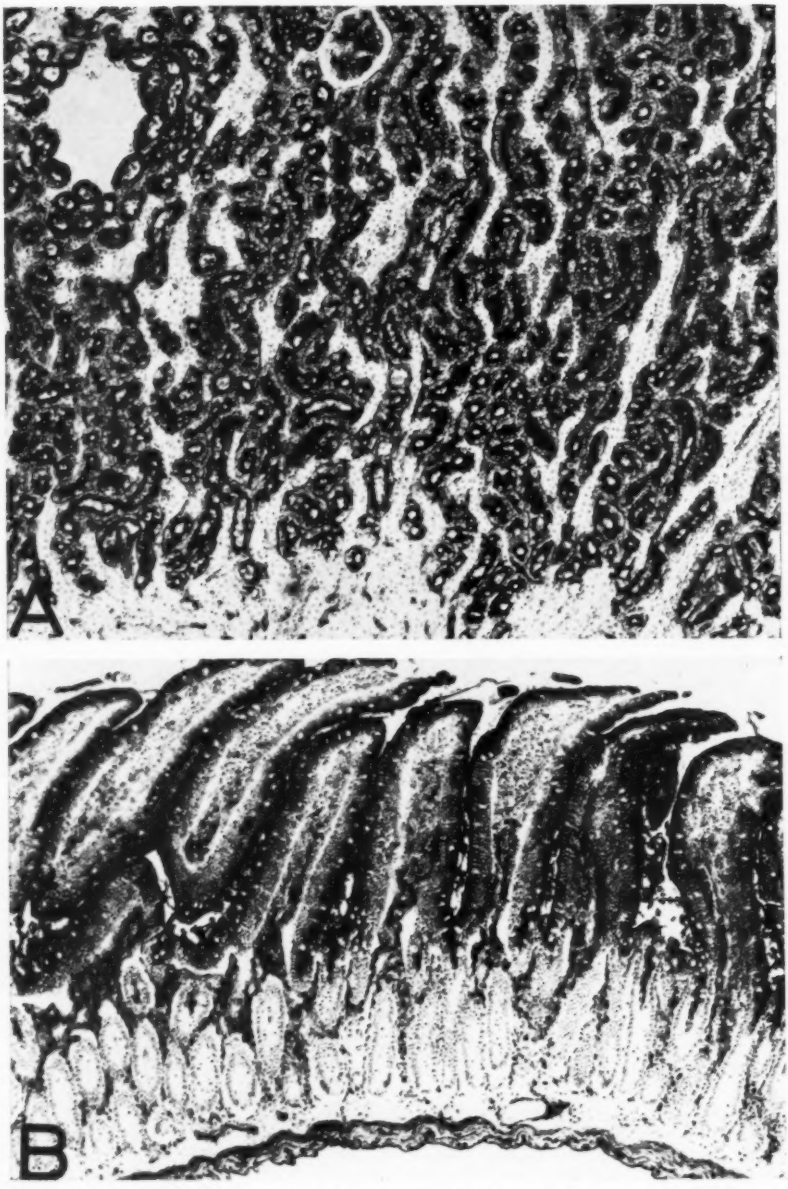

FIGURE 9.—Gomori's method for alkaline phosphatase. × 90. *A*, Kidney of rat bearing a fifteenth-generation transplant. Abundance of enzyme in glomerulus and convoluted tubules of cortex. *B*, Intestine. Concentration of phosphatase in superficial two-thirds of mucosa.

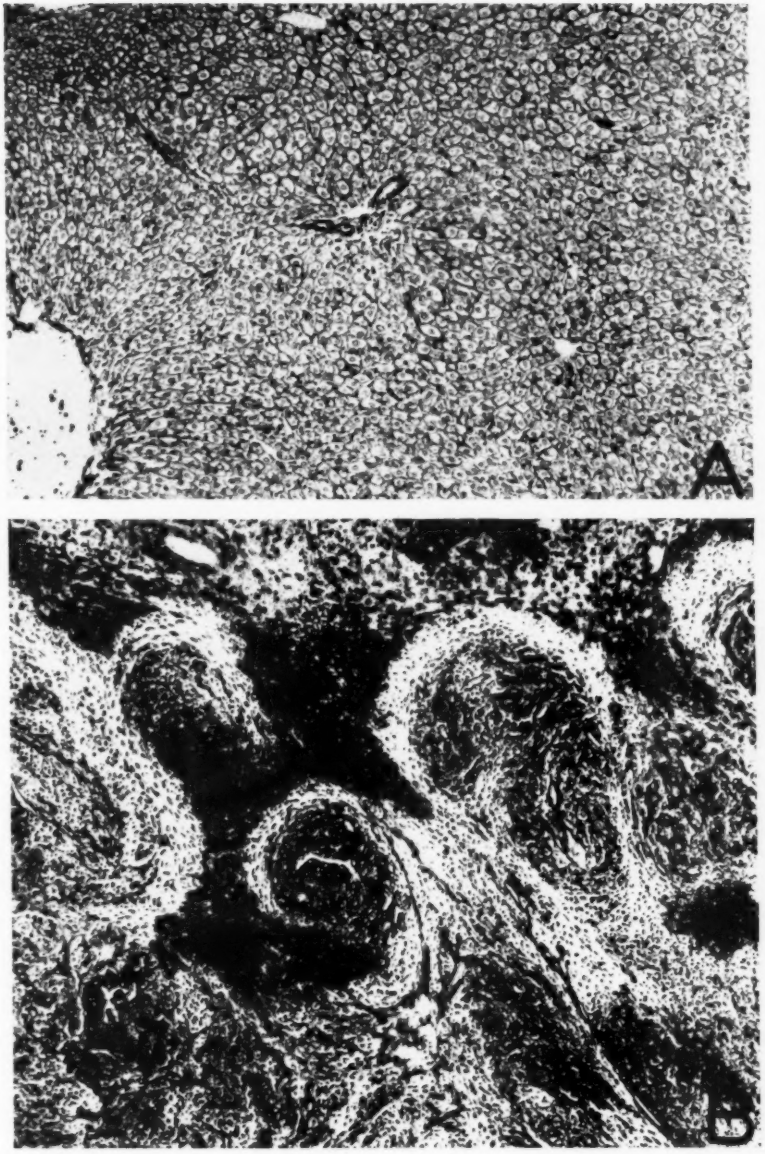

FIGURE 10.—Alkaline phosphatase. × 90. *A*, Liver of rat shown in figure 9. Relatively little enzyme. Note concentration at cell margins. *B*, Fifteenth-generation transplant. Viable cells contain enzyme. Particular concentration in necrotic zones.

The blood supply to the tumors was scanty and present in relatively few capillaries which were very closely applied to neighboring tumor cells, often being separated by only a strand of reticulum. In all except the first two generations those tumors 1 cm. or more in diameter showed central necrosis. The pattern of distribution of the necrotic tissue was constant in the various generations. In the zones of necrosis there were evenly scattered foci of viable cells surrounding centrally placed narrow blood vessels (fig. 11). The areas of necrosis progressively showed hemorrhage, infiltrating leukocytes, removal of necrotic material, calcification of debris, and finally fibrosis.

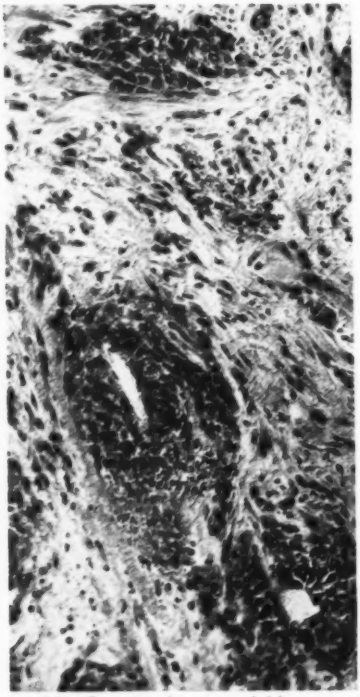

FIGURE 11.—In necrotic zones viable tumor cells persist about blood vessels. The necrotic cells have been removed, and the remaining stroma is increased in density. Twelfth generation. Haematoxylin and eosin. ×150.

In the third generation, microscopic examination of a pretracheal mediastinal lymph node showed hepatoma within the peripheral sinus and also invading and partly replacing the substance of the node (fig. 12).

DISCUSSION

The major point for consideration is the cell of origin of the tumor. Although it is impossible to determine just which of the types of neoplasm were transplanted from the primary tumor tissue, it appears that the hepatic parenchymal cell is the cell of origin in the transplants. The features which support this idea are the acidophilic cytoplasm, the prominent cell membranes, the large vesicular nuclei, prominent nucleoli, subdivision of the tumor into cords often lying parallel to each other, and the characteristic liverlike association of the blood vessels to the epithelium.

The presence of acini in the tumor is entirely consistent with the diagnosis of hepatoma, namely, a tumor derived from the hepatic parenchymal cell. In the primary tumor tissue there were acinar formations in areas of unquestioned hepatoma. Furthermore, we have shown in earlier studies that hepatomas can and not infrequently do exhibit acinar structures (*10*).

The pictures exhibited by the mitochondria and Golgi apparatus are very similar to those seen in mouse hepatomas induced with carbon tetrachloride or *o*-aminoazotoluene, whether primary or transplanted (*13*).

The presence of bone in hepatic tumors of the rat is not common. In an earlier communication (*10*) we reported two instances of membranous bone formation in primary butter-yellow-induced carcinoma of the liver. In one of these hyalin cartilage was shown as well. As far as we can determine, these are the only two cases recorded for the rat in which bone was

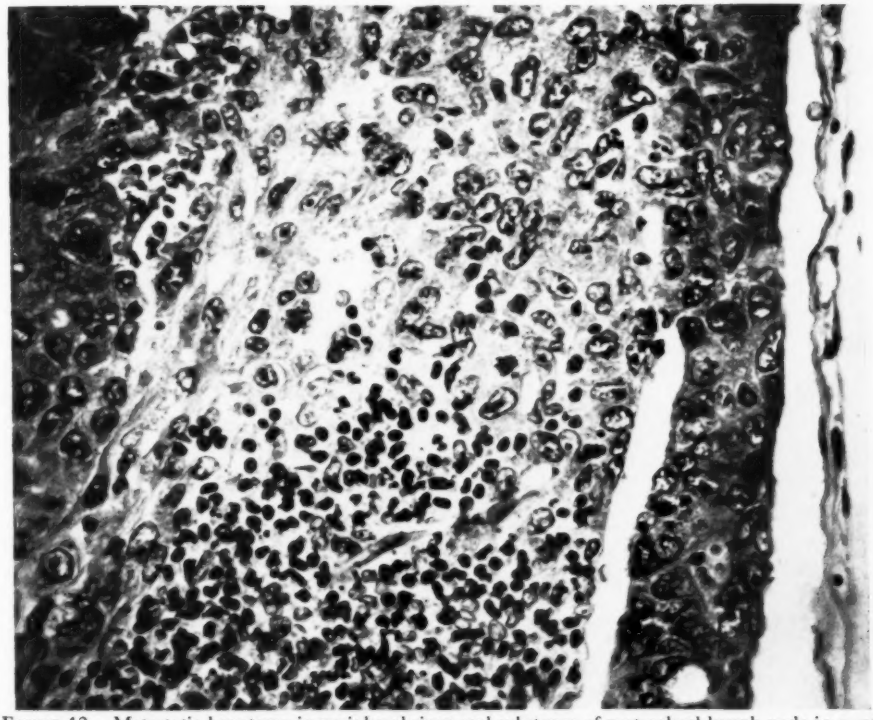

FIGURE 12.—Metastatic hepatoma in peripheral sinus and substance of pretracheal lymph node in a rat bearing a third-generation subcutaneous transplant. Haematoxylin and eosin. × 450.

found to be growing in the stroma of induced hepatic tumors. The current case appears to be the only one in which membranous bone was found in an induced transplanted hepatoma in this animal. The mechanism of such bone formation is unknown, but the presence of considerable alkaline phosphatase in the tumor here reported is an interesting and, perhaps, an important association. This may be analogous to the results of Huggins who, in dogs, obtained bone formation about urinary bladder mucosa transplanted to the rectus sheath (*14*).

In the paper of Kabat and Furth (*15*) on the distribution of alkaline phosphatase as determined by the staining technique, this enzyme was reported in a primary induced hepatic carcinoma of the rat.

This finding in a primary tumor is in keeping with those in the transplanted hepatoma here recorded. Greenstein (*7*) in studying our tumor observed an abundance of alkaline phosphatase by direct quantitative determinations, and we have demonstrated a considerable amount of it in sections stained for this enzyme.

In contrast with these observations on the hepatoma of the rat, the quantitative studies of Greenstein (*7*) and stains made by the present authors have demonstrated that mouse hepatomas, primary or transplanted, either arising spontaneously (*16*) or induced with *o*-aminoazotoluene or carbon tetrachloride (*17*), show low values for alkaline phosphatase. The facts presented suggest that the difference between the values for this enzyme in hepatomas

of the rat and the mouse depends on a species influence rather than on whether the tumor is spontaneous or induced, primary or transplanted.

Support to such a hypothesis is given by studies on glycogen in the hepatic tumors. With Best's carmine stain, glycogen was absent from cells of the tumor here recorded as well as from the cells of other representative induced primary and transplanted rat hepatomas (*10*). In contrast, an abundance of this substance was observed in mouse hepatomas transplanted and primary, and spontaneous and induced (*16–18*).

SUMMARY

The history and pathology of rat hepatoma 31 are presented. The tumor, a malignant hepatoma, arose in the liver of an Osborne-Mendel rat fed butter yellow. It is readily transplantable in the homologous strain of rat and is now in the sixteenth generation. It is suitable for studies in which liver and a tumor of hepatic parenchymal cell origin are to be compared.

REFERENCES

(*1*) Greenstein, J. P., Jenrette, W. V., Mider, G. B., and White, J.: Chemical studies on the components of normal and neoplastic tissues. V. The relative arginase activity of certain tumors and normal control tissues. J. Nat. Cancer Inst., 1: 687–706 (1941).

(*2*) Greenstein, J. P., and Jenrette, W. V.: The depolymerization of thymonucleic acid by an enzyme system in normal and cancerous hepatic and mammary tissues and in the milk and sera of several species. J. Nat. Cancer Inst., 1: 845–863 (1941).

(*3*) Greenstein, J. P., Jenrette, W. V., and White, J.: The relative activity of xanthine dehydrogenase, catalase, and amylase in normal and cancerous hepatic tissues of the rat. J. Nat. Cancer Inst., 2: 17–22 (1941).

(*4*) Maver, M. E., Mider, G. B., Johnson, J. M., and Thompson, J. W.: The comparative proteinase and peptidase activities of rat hepatoma and normal and regenerating rat liver. J. Nat. Cancer Inst., 2: 277–282 (1941).

(*5*) Greenstein, J. P., Jenrette, W. V., and White, J.: Note on the composition of the nucleoprotein fraction of normal liver and of the transplanted hepatic tumor in the rat. J. Nat. Cancer Inst., 2: 305–306 (1941).

(*6*) Greenstein, J. P., Jenrette, W. V., and White, J.: The liver catalase activity of tumor-bearing rats and the effect of extirpation of the tumors. J. Nat. Cancer Inst., 2: 283–291 (1941).

(*7*) Greenstein, J. P.: The distribution of acid and alkaline phosphatase in tumors, normal tissues, and the tissues of tumor-bearing rats and mice. J. Nat. Cancer Inst., 2: 511–524 (1942).

(*8*) Robertson, W. B., and Kahler, H.: Riboflavin content of tumor tissues. J. Nat. Cancer Inst., 2: 595–600 (1942).

(*9*) White, A., and Jackson, R. W.: The effect of bromobenzene on the utilization of cystine and methionine by the growing rat. J. Biol. Chem., 111: 507–513 (1935).

(*10*) Edwards, J. E., and White, J.: Pathologic changes, with special reference to pigmentation and classification of hepatic tumors, in rats fed *p*-dimethylaminoazobenzene (butter-yellow). J. Nat. Cancer Inst., 2: 157–183 (1941).

(*11*) Steffens, H. W.: An experimental study of the mitochondria in the hepatic cells of the white rat. Anat. Rec., 81: 243–263 (1941).

(*12*) Gomori, G.: Microchemical demonstration of phosphatase in tissue sections. Proc. Soc. Exper. Biol. & Med., 42: 23–26 (1939).

(*13*) Dalton, A. J., and Edwards, J. E.: Mitochondria and Golgi apparatus of induced and spontaneous hepatomas in the mouse. J. Nat. Cancer Inst., 2: 565–575 (1942).

(*14*) Huggins, C. B.: The formation of bone under the influence of epithelium of the urinary tract. Arch. Surg., 22: 377–408 (1931).

(*15*) Kabat, E. A., and Furth, J.: A histochemical study of the distribution of alkaline phosphatase in various normal and neoplastic tissues. Am. J. Path., 17: 303–318 (1941).

(*16*) Edwards, J. E., Dalton, A. J., and Andervont, H. B.: Pathology of a transplantable spontaneous hepatoma in a C3H mouse. J. Nat. Cancer Inst., 2: 555–563 (1942).

(*17*) Edwards, J. E. and Dalton, A. J.: Induction cirrhosis of the liver and of hepatomas in mice with carbon tetrachloride. J. Nat. Cancer Inst., 3, No. 1 (August 1942).

(*18*) Strong, L. C., and Smith, G. M.: Preuve histologique de la présence de glycogène dans un hepatoma malin transplantable. Bull. Assoc. franç. p. l'étude du cancer, 26: 694–698 (1937).

Pathology of a Transplantable Spontaneous Hepatoma in a C3H Mouse[1]

By Jesse E. Edwards, *research fellow*, Albert J. Dalton, *cytologist*, and Howard B. Andervont, *principal biologist, National Cancer Institute, National Institute of Health, United States Public Health Service*

In this paper a transplantable hepatoma arising spontaneously in a C3H male mouse is reported. It appears pertinent to record this case for several reasons. There is a paucity of literature on transplantable spontaneous hepatoma in the mouse and apparently no report on such a tumor in the C3H strain. Study of the Golgi apparatus here reported indicates that the spontaneous hepatoma, hitherto considered nonneoplastic by some, is indeed a neoplasm. The tumor is designated as mouse hepatoma 98/15 and is being carried in transplant in suitable animals at the National Cancer Institute.

HISTORY OF TUMOR

The tumor arose in the liver of a 17-months-old male mouse belonging to the Andervont line of the C3H strain. The animal had been nursed by its own mother and after weaning had been maintained on a diet of Purina dog-chow pellets. The animal had received no treatment and was apparently in good health when killed.

At autopsy, September 12, 1940, pieces of the tumor were transplanted by the trocar technique to the subcutaneous tissue of four male mice of the homologous strain and line. Of these, one showed a transplant 1.0 cm. in diameter in May 1941, 8 months later. The second mouse presented a similar tumor in October 1941, 13 months after transplantation, and the two remaining recipients were negative for tumor at 15 months when they were discarded. Transplantation has been continued from each of the two tumor lines obtained, the first now in the fifth generation, and the second in the third generation. The rate of growth of the original passage was slower than that of the more recent transplants which now reach a diameter of 1.0 cm. in about 2 months.

PATHOLOGY

Gross Examination

Primary Tumor

The primary growth was situated in one of the major lobes of the liver and was a globular, solitary, discrete, soft, pale-yellow mass measuring 1.2 cm. in diameter and covered by a smooth, intact capsule. The liver removed from the tumor as well as the other organs showed no abnormality. No metastases or peritoneal implants were observed.

Transplants

All the transplants were essentially similar in appearance, presenting lobulated, yellowish-gray outer surfaces which were securely attached to the adjacent subcutaneous tissues. The transplant tissue was soft, friable, and on section appeared yellowish-pink and moist with blood oozing from the cut surface. No lymph node or visceral metastases have been found in any mice carrying transplants.

[1] *Presented before the annual meeting of the American Association of Pathologists and Bacteriologists, St. Louis, April 1942, under the title "A Transplantable Spontaneous Hepatoma in a Strain C3H Mouse."*

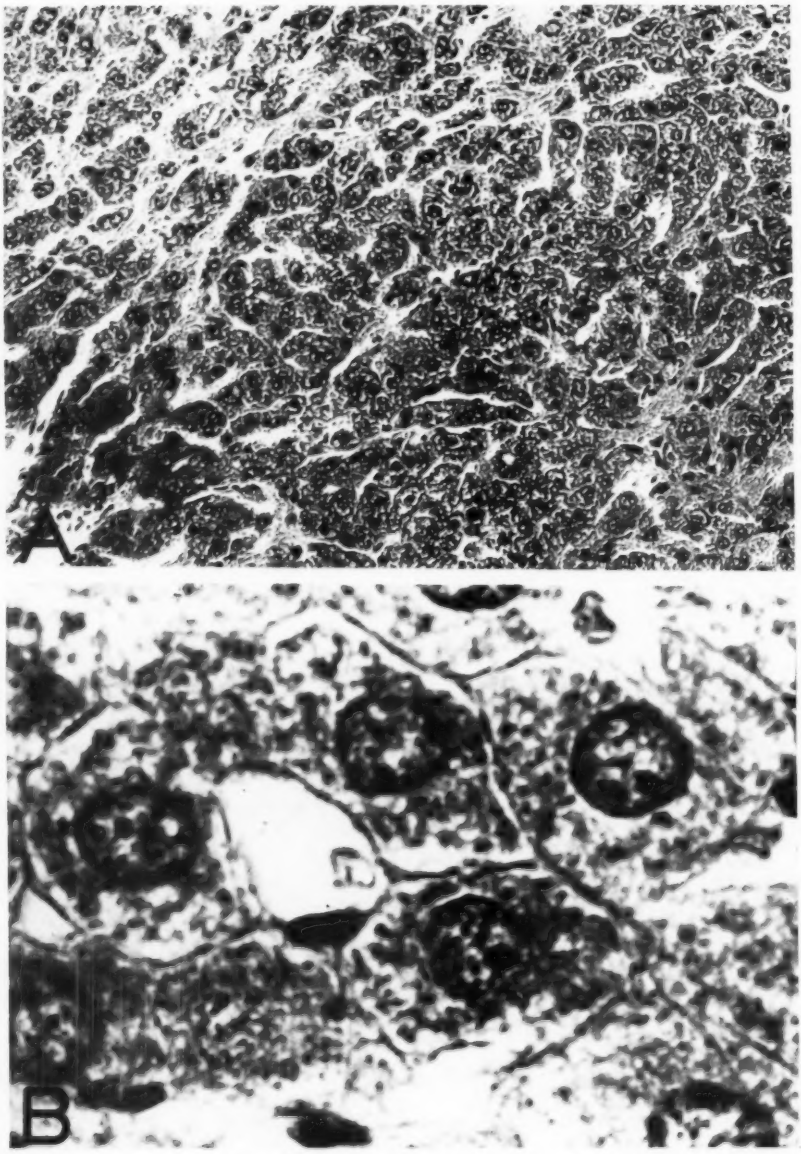

FIGURE 1.—Primary tumor. Haematoxylin and eosin stain. *A*, × 210; *B*, × 1760.

Microscopic Examination

Primary Tumor

The primary growth had the structure of a very well differentiated hepatoma with cords of epithelial cells alternating regularly with endothelial-lined blood sinuses of varying diameter. The epithelial cells had a striking resemblance to hepatic parenchymal cells with well-defined cytoplasmic membranes, granular cytoplasm, and relatively large vesicular nuclei containing one to three prominent, large, basophilic nucleoli (fig. 1).

With the haematoxylin and eosin stain, the tumor cells could be distinguished from those of the liver by their greater size and faintly basophilic cytoplasm. A few mitotic figures were present in the neoplasm. There was no true lobular arrangement of the epithelial cords, and the few bile ducts present were distributed haphazardly and almost always at the periphery of the tumor. No pigment granules were found within the tumor cells.

Globular bodies about the size of the average nucleus were scattered unevenly through the growth usually within the cytoplasm of the epithelial cells (fig. 2). As a rule these were acidophilic, but occasionally they were basophilic or faintly green with the haematoxylin and eosin stain. When stained with Mallory's phosphotungstic acid haematoxylin they were reddish-brown, and with the aniline blue connective tissue stain they presented a central blue core, an intermediate unstained zone, and a blue margin.

The liver removed from the tumor showed no microscopic abnormality. Of particular interest was the absence of increased connective tissue when examined with Mallory's aniline blue connective tissue and phosphotungstic acid haematoxylin stains.

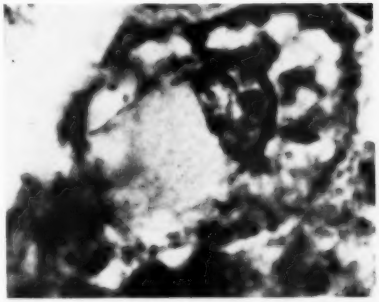

Figure 2.—Globular inclusion in cytoplasm of a hepatoma cell in a first-generation transplant similar to those seen in the primary tumor. Mallory's phosphotungstic acid haematoxylin stain. ×1760.

Transplants

The transplants were invasive and not encapsulated and presented a structure essentially similar to the primary tumor (fig. 3). There was, however, a greater number of mitotic figures. A considerable amount of fat was demonstrated in the early generations by the osmic acid and sudan IV stains for this substance. Later generations possessed less.

Glycogen, as demonstrated by Best's carmine stain, was abundant in the cytoplasm in all generations. Occasionally cells were encountered which contained large vacuoles giving the reaction for neither fat nor glycogen. This condition may have represented hydropic change. As in the primary tumor, epithelial cells were devoid of pigment granules, but a few phagocytes, usually at the periphery of the growth, contained yellowish-brown granules which gave a positive reaction for iron. The globular hyalin bodies (fig. 2) described in the original tumor were seen in the various transplants, often in large numbers. Representative sections of transplants and livers of the tumor-bearing mice stained for alkaline phosphatase by the method of Gomori (*1*) revealed little or none in the epithelial

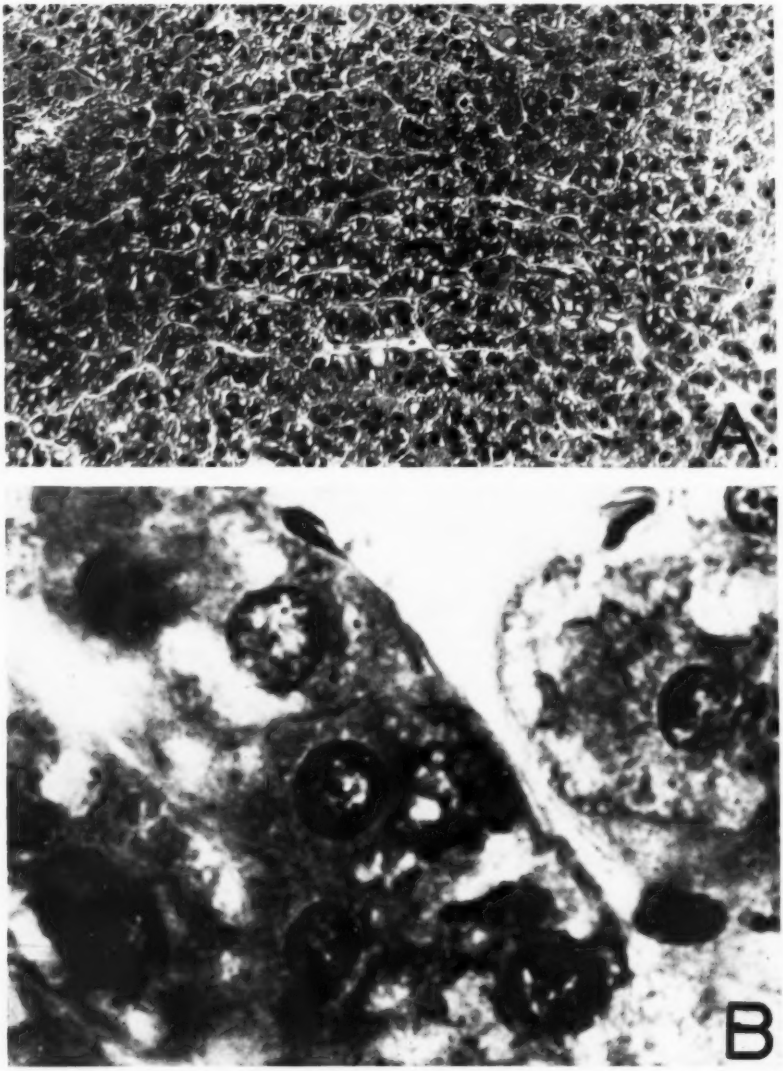

FIGURE 3.—Third-generation transplant of spontaneous hepatoma pictured in figure 1. Haematoxylin and eosin stain. *A*, × 210; *B*, × 1760.

cells, while the intestinal mucosa and kidneys of the same animals contained an abundance of this enzyme.

The peripheral distribution of the Golgi apparatus found in normal hepatic cells was absent here. Instead, the Golgi apparatus of cells of the transplants was an undifferentiated, juxtanuclear, condensed network (fig. 4). This latter type of Golgi apparatus is similar to that found

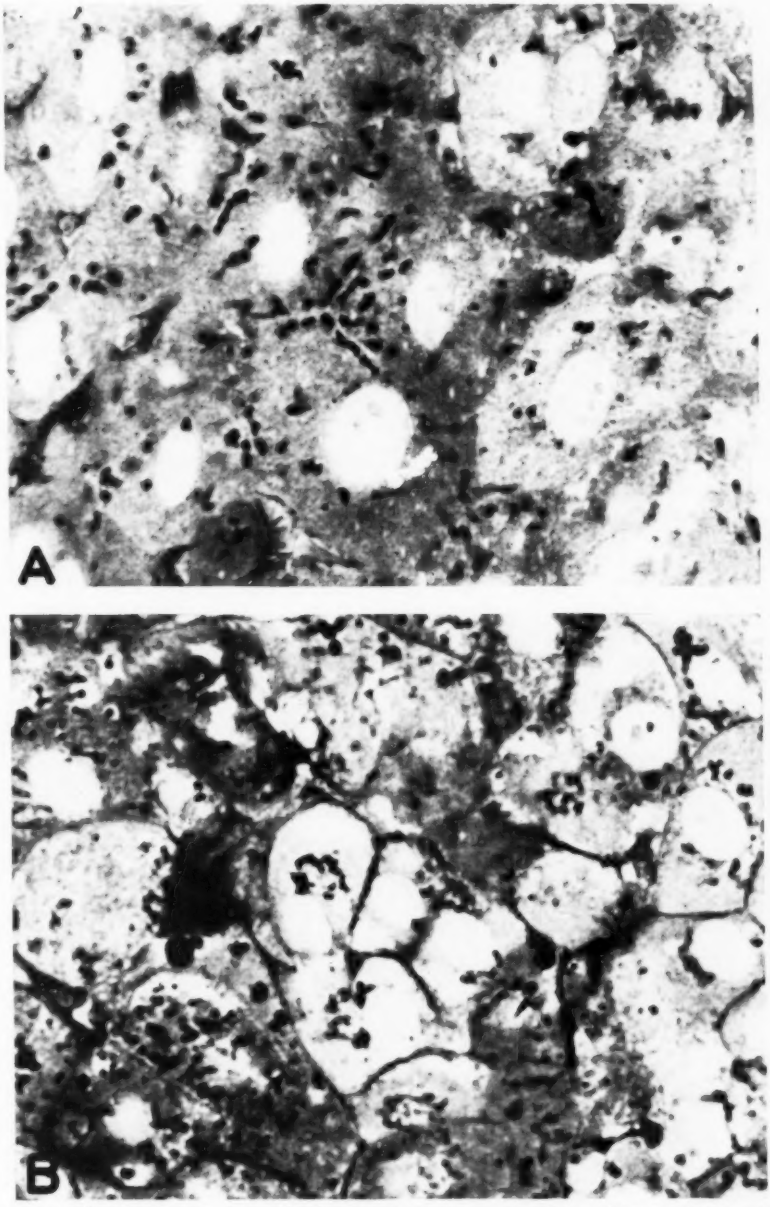

FIGURE 4.—*A*, Normal pattern of the Golgi apparatus in the mouse liver. Note partially peripheral distribution. Kolatchew-Nassonow method. × 1300; *B*, Golgi apparatus of cells of hepatoma 98/15. Note its juxtanuclear position. Kolatchew-Nassonow method. × 1300.

in induced hepatomas in the mouse and rat (*6*). In normal cells the mitochondria vary from spheres and plump rods at the periphery of the lobule to long filaments

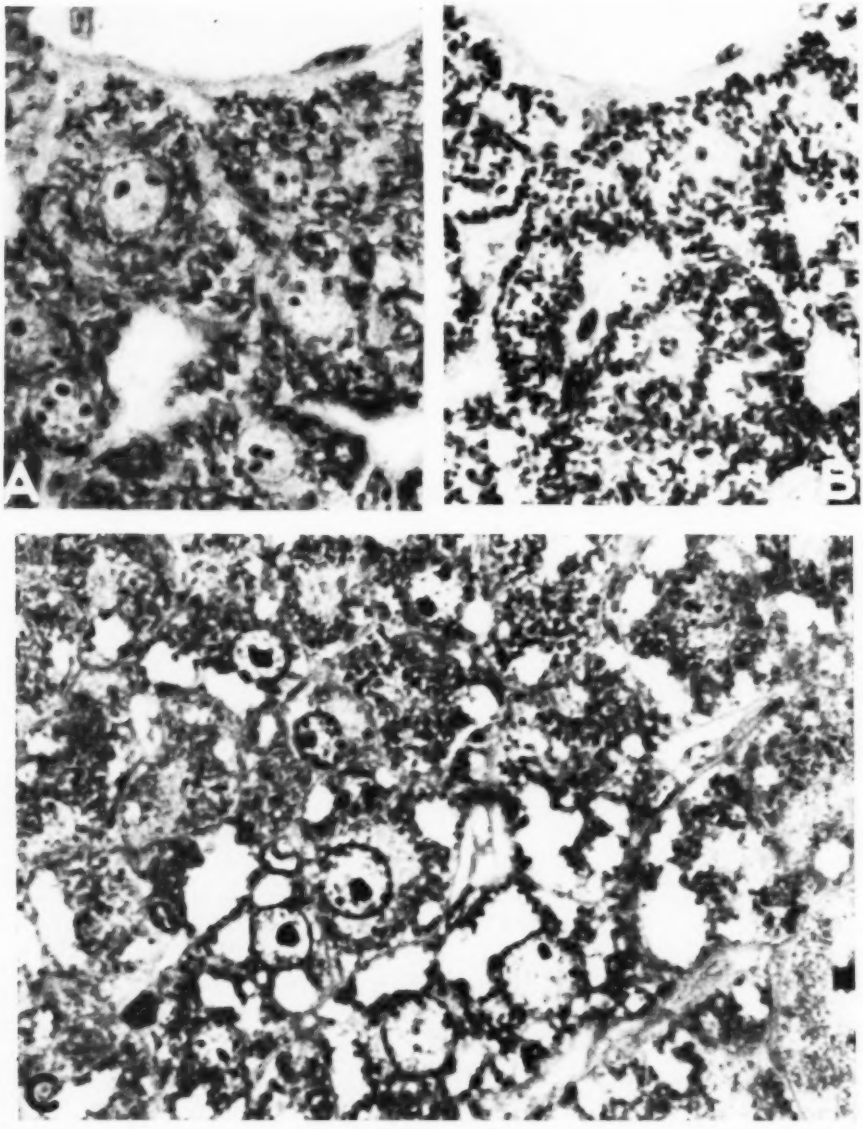

FIGURE 5.—*A*, Filamentous mitochondria in hepatic cells of normal mouse liver near central vein. Regaud fixation. Iron haematoxylin stain. × 1300; *B*, Spherical mitochondria in normal hepatic cells near periphery of lobule. Regaud fixation. Iron haematoxylin stain. × 1300; *C*, Spherical mitochondria in hepatoma 98/15. Regaud fixation. Iron haematoxylin stain. × 1300.

in cells near the central vein. The mitochondria of the cells in the transplants appeared consistently as medium-sized spheres (fig. 5). Other primary spontaneous hepatomas studied have shown exactly the same picture.

DISCUSSION

As indicated in the introduction to this paper, the literature reveals little in the way of reports on successful transplantation of spontaneous hepatomas. We were able to find only one report of a transplantable spontaneous hepatoma in the mouse, in an animal of the CBA strain (*2*). Burns and Schenken (*3*) attempted to transplant spontaneous hepatomas in the C3H strain of mouse. After 2 to 7 months, however, they reported having no success in their animals still under observation when their paper was written. The long incubation period of transplants of spontaneous hepatomas may explain the lack of many reports of successful takes. Many mice have probably been discarded by some investigators before sufficient time has elapsed for growth of transplants to become evident. In the present work it required as long as 13 months for one of the transplants to reach a diameter of 1.0 cm. In contrast with the slow rate of growth in this tumor, we have under observation a transplantable spontaneous hepatoma in a strain A mouse which grew to a diameter of 1.0 cm. within 3 months from the time of the original transplantation.

The nature of the so-called spontaneous hepatoma of the mouse requires some consideration. Two different interpretations present themselves, each with its adherents: One is that the lesion is not a neoplasm, being either a hyperplastic nodule of regenerated tissue or a developmental anomaly. The other interpretation is that it is, indeed, a neoplasm. The arguments used to support the nonneoplastic nature of the growth are the high degree of differentiation of the cells together with their definite tendency to form liverlike cords alternating with endothelial-lined sinuses, the presence of bile ducts in the primary mass, and the capacity for storing fat and glycogen (*4*).

That the lesion is regenerative in character is difficult to reconcile with the absence of any demonstrable damage in liver removed from the hepatoma, and the fact that this lesion is seen in mice consistently after middle age rather than in young ones contradicts the idea of a congenital anomaly. Moreover, the fact that the cells of the lesion are very well differentiated does not rule out the possibility of neoplasm. In cases of human beings with hepatoma, it is not uncommon to find the primary tumor and metastases exhibiting an extremely high degree of differentiation. Similarly, in rats fed butter yellow one often finds blood-vessel invasion by extremely well differentiated hepatomas histologically comparable with the spontaneous hepatoma in the mouse (*5*). Moreover, while the cells of the lesion under discussion resemble in many ways the hepatic parenchymal cells, the Golgi apparatus is profoundly dissimilar to that of the normal liver as well as the liver regenerating from partial hepatectomy or chemical injury (*6*). Indeed, the Golgi apparatus of the spontaneous mouse hepatoma is virtually indistinguishable from that of induced hepatomas in mice and rats. This distinctive arrangement and position of the Golgi apparatus which we have found characteristic for hepatoma is considered by Cramer and Ludford (*7*) to indicate the resting stage of an exocrine secretory cycle. Thus, this type of Golgi apparatus, suggestive of exocrine inactivity, may have its corollary in the absence of pigment in the hepatoma cell. The mitochondrial

pattern, while different from that of induced hepatomas, is also distinctly different from that of normal or regenerating liver.

The liverlike formation of epithelial cords alternating with endothelial-lined blood sinuses is a feature exhibited not only by the spontaneous hepatoma of the mouse but also by the induced hepatomas in this animal as well as by metastasizing primary carcinomas of the liver in the rat (*5*), cow (*8*), man, and other species. This fundamental architectural resemblance to the normal liver cannot, therefore, be used to support the idea of a nonneoplastic nature of the spontaneous mouse hepatoma.

The bile ducts found in the spontaneous hepatoma are of interest. Microscopically these are very well differentiated and indistinguishable from the bile ducts in the portal canals of the liver. Explanation of their presence may be attempted in two ways, (1) that they represent a peculiar differentiation of the tumor cells, or (2) that they are mere inclusions in the tumor of pre-existing ducts (*3*). The latter view would seem more acceptable since these structures are seen only in primary hepatomas and not in transplanted ones. Moreover, the presence of bile ducts in hepatic tumors is not restricted to the hepatoma, for bile ducts are seen in nonepithelial primary tumors of the liver, for example, the hemangioendothelioma, and in a variety of metastatic tumors growing in this organ.

The presence of fat and glycogen (*4*) in the epithelial cells of the spontaneous hepatoma is not unique since we have also found this in primary and in transplanted *o*-aminoazotoluene- and carbon tetrachloride-induced hepatomas in the mouse (*9*). The glycogen content of hepatoma cells seems to depend on species rather than on whether the lesion is spontaneous or induced. In the rat, the cells of the primary and transplanted butter-yellow-induced hepatomas are devoid of stainable glycogen even after forced feeding of glucose (*5*). Similar differences are reported in hepatomas of man (*10*) and the cow (*8*), since the former does not show glycogen and the latter does.

On the basis of the evidence presented, it would appear that the spontaneous hepatoma is neoplastic in nature, having many features in common with accepted hepatic tumors of other species and with induced hepatomas in the mouse. Whether it is malignant or not is more difficult to decide. While metastases have been reported (*11*, *12*), they must be extremely unusual. We have observed neither metastases nor invasion of blood vessels in a large number of mice with spontaneous hepatomas. Furthermore, large hepatomas do not appear to have any deleterious effect on the health of their hosts. Transplantability, per se, cannot be considered an absolute indication of malignancy because a benign tumor such as the mammary fibroadenoma of the rat may also be transplantable. Recently, Andervont and Shimkin (*13*) have had success with intrapulmonary transplantation of apparently normal gastric glandular mucosa in mice of strains C, dba, and C57 Black, as well as of the hyperplastic but probably nonneoplastic gastric lesion of strain I mice. It is, therefore, considered for the time being, at least, that the question as to whether the spontaneous hepatoma of the mouse is benign or malignant remains open.

The absence of stainable alkaline phosphatase in transplants of the tumor presented is in line with results of studies on this enzyme in induced hepatomas in the mouse. We obtained similarly negative results in primary carbon tetrachloride-induced hepatomas (*9*). Greenstein (*14*),

doing quantitative investigations, reported very low values for alkaline phosphatase in transplanted *o*-aminoazotoluene-induced hepatomas in the mouse. These observations are in marked contrast with the high values for alkaline phosphatase obtained with butter-yellow-induced hepatomas in the rat studied by both quantitative methods and staining techniques (*14*, *15*, *16*).

These studies suggest that, as with glycogen storage, the values for alkaline phosphatase in a hepatoma depend upon species rather than upon whether the tumor is spontaneous or induced.

SUMMARY AND CONCLUSIONS

Studies of a transplantable hepatoma arising spontaneously in a male mouse of the C3H strain are presented. The primary tumor and the transplants exhibited the structure of a well-differentiated hepatoma. The tumor cells of the transplants contained fat and gylcogen but little or no stainable alkaline phosphatase. The Golgi apparatus of the cells was similar to that in induced hepatomas of the mouse and rat and differed from that in the parenchymal cells of normal and regenerating liver. The mitochondrial pattern differed both from that in the cells of normal liver and induced hepatomas.

The weight of evidence indicates that the spontaneous hepatoma of the mouse is a neoplasm, but whether it is benign or malignant is as yet an unsettled matter.

REFERENCES

(*1*) GOMORI, G.: Microchemical demonstration of phosphatase in tissue sections. Proc. Soc. Exper. Biol. & Med., 42: 23–26 (1939).

(*2*) STRONG, L. C., and SMITH, G. M.: Successful transplantation of a hepatoma in mice. Am. J. Cancer, 28: 112–114 (1936).

(*3*) BURNS, E. L., and SCHENKEN, J. R.: Spontaneous primary hepatomas in mice of strain C_3H. A study of incidence, sex distribution and morbid anatomy. Am. J. Cancer, 39: 25–35 (1940).

(*4*) STRONG, L. C., and SMITH, G. M.: Preuve histologique de la présence de glycogène dans un hepatoma malin transplantable. Bull. Assoc. franç. p. l'étude du cancer, 26: 694–698 (1937).

(*5*) EDWARDS, J. E., and WHITE, J.: Pathologic changes, with special reference to pigmentation and classification of hepatic tumors in rats fed *p*-dimethylaminoazobenzene (butter yellow). J. Nat. Cancer Inst., 2: 157–183 (1941).

(*6*) DALTON, A. J., and EDWARDS, J. E.: Mitochondria and Golgi apparatus of induced and spontaneous hepatomas in the mouse. J. Nat. Cancer Inst., 2: 565–575 (1942).

(*7*) CRAMER, W., and LUDFORD, R. J.: On the cellular mechanism of bile secretion and its relation to the Golgi apparatus of the liver cell. J. Physiol., 62: 74–80 (1926).

(*8*) FELDMAN, W. H.: Primary carcinoma of the liver. Two cases in cattle. Am. J. Path., 4: 593–600 (1928).

(*9*) EDWARDS, J. E., and DALTON, A. J.: Induction of cirrhosis of the liver and of hepatomas in mice with carbon tetrachloride. J. Nat. Cancer Inst., 3, No. 1 (August 1942).

(*10*) LUBARSCH, O.: Über die Bedeutung der pathologischen Glykogenablagerungen. Arch. f. path. Anat. u. Phys. u. f. klin. Med., 183: 188–228 (1906).

(*11*) SLYE, M., HOLMES, H. F., and WELLS, H. G.: Spontaneous primary tumors of the liver in mice. Studies on the incidence of inheritability of spontaneous tumors in mice. J. M. Research, 33: 171–182 (1915).

(*12*) GORER, P. A.: The incidence of tumours of the liver and other organs in a pure line of mice (Strong's CBA strain). J. Path. & Bact., 50: 17–24 (1940).

(*13*) ANDERVONT, H. B., and SHIMKIN, M. B.: Intrapulmonary transplantation of the adenomatous gastric lesion of strain I mice. J. Nat. Cancer Inst., 2: 151–155 (1941).

(*14*) GREENSTEIN, J. P.: Distribution of acid and alkaline phosphatase in tumors, normal tissues, and the tissues of tumor-bearing rats and mice. J. Nat. Cancer Inst., 2: 511–524 (1942).

(*15*) KABAT, E. A., and FURTH, J.: A histochemical study of the distribution of alkaline phosphatase in various normal and neoplastic tissues. Am. J. Path., 17: 303–318 (1941).

(*16*) WHITE, J., DALTON, A. J., and EDWARDS, J. E.: Pathology of rat hepatoma 31. J. Nat. Cancer Inst., 2: 539–554 (1942).

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Mitochondria and Golgi Apparatus of Induced and Spontaneous Hepatomas in the Mouse

By ALBERT J. DALTON, *cytologist*, and JESSE E. EDWARDS, *research fellow*, *National Cancer Institute, National Institute of Health, United States Public Health Service*

Many attempts have been made to discover precise morphologic criteria for distinguishing between neoplastic cells and the normal cell type from which they arise. In these attempts several studies of the mitochondria and the Golgi apparatus of tumor cells have been made by different investigators. As indicated by Cowdry (*1*), they have been for the most part negative or contradictory. For example, Bagozzi (*2*) concluded from a study of nine different human tumors that there was nothing abnormal in the structure of the Golgi apparatus in tumor cells. He suggested that comparison of mitochondrial forms might be of some value (*3*). It should be pointed out, however, that few direct comparisons have been made between neoplastic cells and the cells from which they arise. This is obviously due in many instances to the great difficulty in establishing the true cell of origin of a tumor with any degree of certainty.

In the present study a series of primary and transplanted, induced and spontaneous hepatomas of the mouse was studied. Neoplasms of the liver were chosen for this investigation since the tumor cell can readily be compared with its cell of origin, namely, the hepatic parenchymal cell (*4*).

MATERIALS AND METHODS

A total of 37 mice of C3H and A strains were used in the experiments. In 1 series 10 mice were killed at intervals during treatment with *o*-aminoazotoluene or CCl_4. In a second series 7 livers were studied in which regenerative changes had been induced by CCl_4 treatment, bile duct ligation, and partial hepatectomy. In a third series 20 mice with spontaneous and induced tumors were studied. Preparations demonstrating mitochondria and the Golgi apparatus were made in each case. The mitochondrial preparations were made by a modification of the Regaud method. Small pieces of tissue were fixed in Regaud's fluid in the cold (2°–3° C.) for 3 days, the fluid being changed each day. After fixation this tissue was postchromated in 5 percent $K_2Cr_2O_7$ at 37° C. for 3 days. Dioxane was used for dehydration followed by paraffin embedding in a vacuum oven. The Golgi apparatus was studied in preparations made by Nassonow's modification of Kolatchew's method—Champy fixation (1 part 2 percent osmic acid, 1 part ¾ percent chromic acid, 1 part 3 percent $K_2Cr_2O_7$) followed by osmication with 2 percent osmic acid at 37°. Five days post osmication was found to be optimum for the impregnation of the Golgi apparatus of normal liver cells, while 4 days usually sufficed for the impregnation of tumor tissue. Alcohol was used for the dehydration of Champy-fixed material since the reduced osmic acid appeared to be slightly soluble in dioxane.

RESULTS

MITOCHONDRIA

Mitochondria of the cells of the normal mouse liver vary from long, tenuous fila-

ments near the central vein (fig. 1, *A*) to spheres and plump rods at the periphery of the lobule (fig. 1, *B*). These observations confirm the detailed ones of Noël (*5*). The mitochondria of all 12 induced hepatomas studied, both primary and transplanted, induced with either CCl_4 or *o*-aminoazotoluene contained only filamentous mitochondria. Figure 2 shows the typical pattern found in CCl_4-induced tumors while figure 3 shows the junction between normal liver and tumor induced

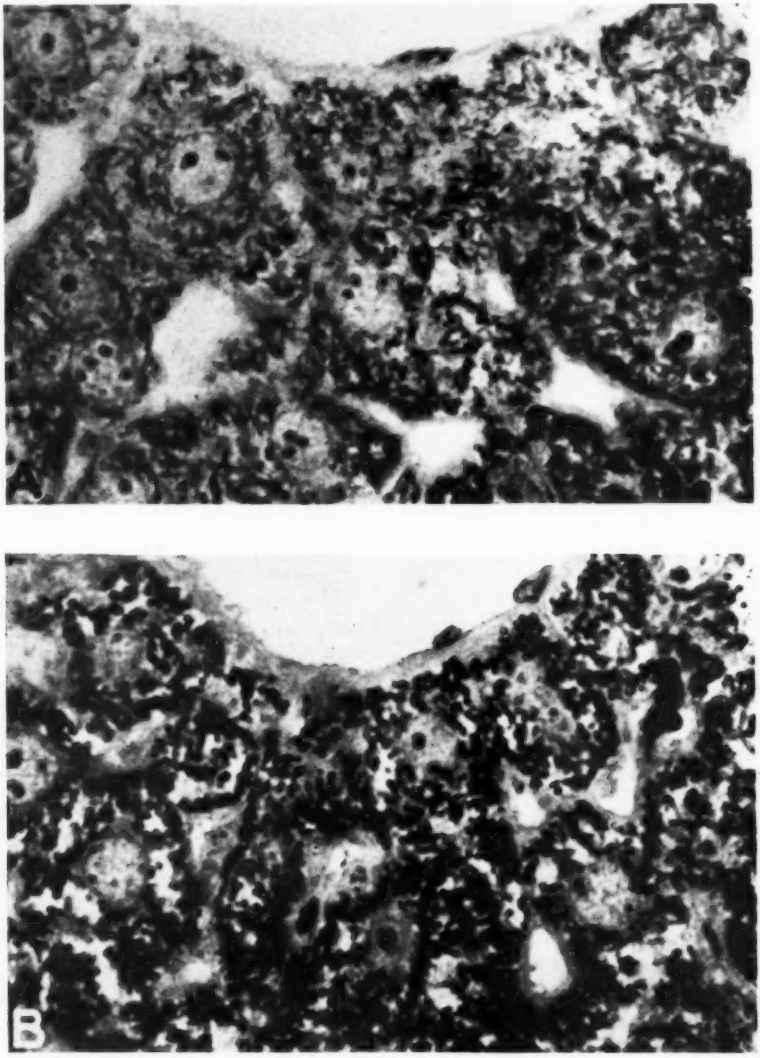

FIGURE 1.—Normal mouse liver. *A*, Near central vein, showing filamentous mitochondria; *B*, Near periphery of lobule, with spherical and short rodlike mitochondria.

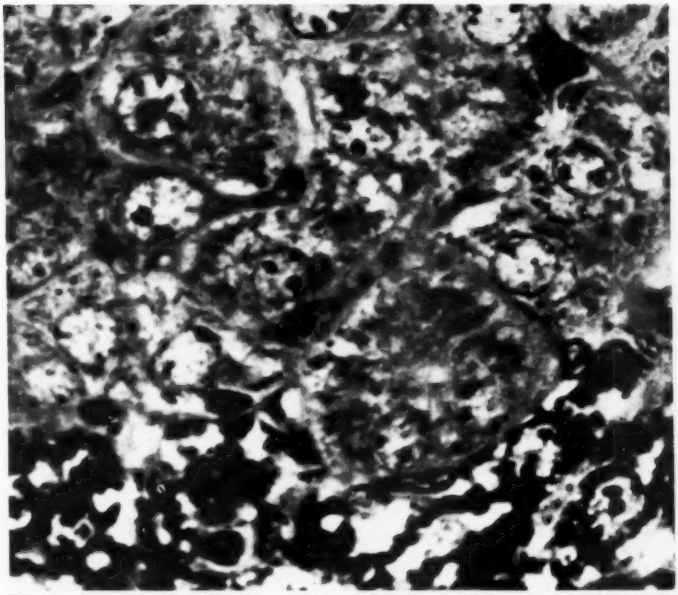

FIGURE 2.—Filamentous mitochondria in a primary hepatoma induced with CCl_4. Tumor above overstained hepatic cells below.

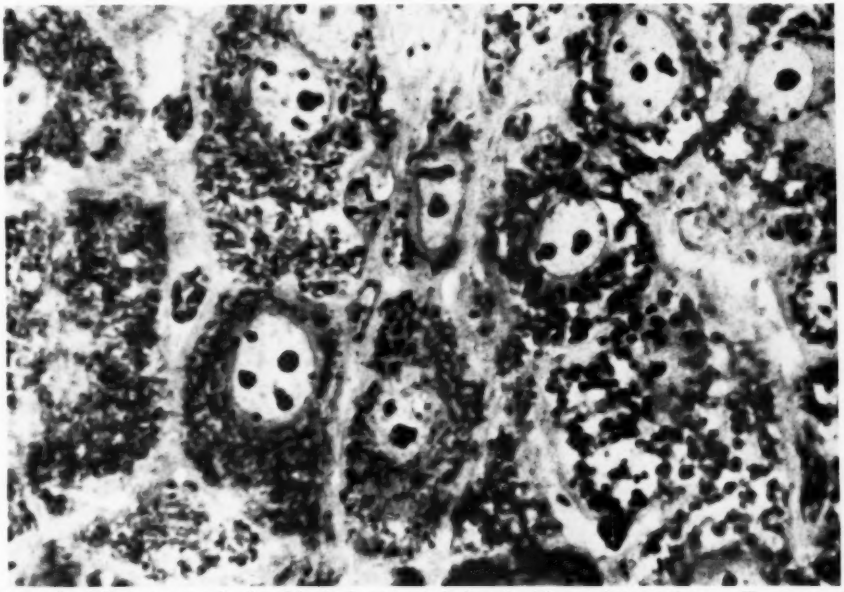

FIGURE 3.—Filamentous mitochondria in hepatoma induced with *o*-aminoazotoluene. Tumor to the left, normal hepatic tissue to the right.

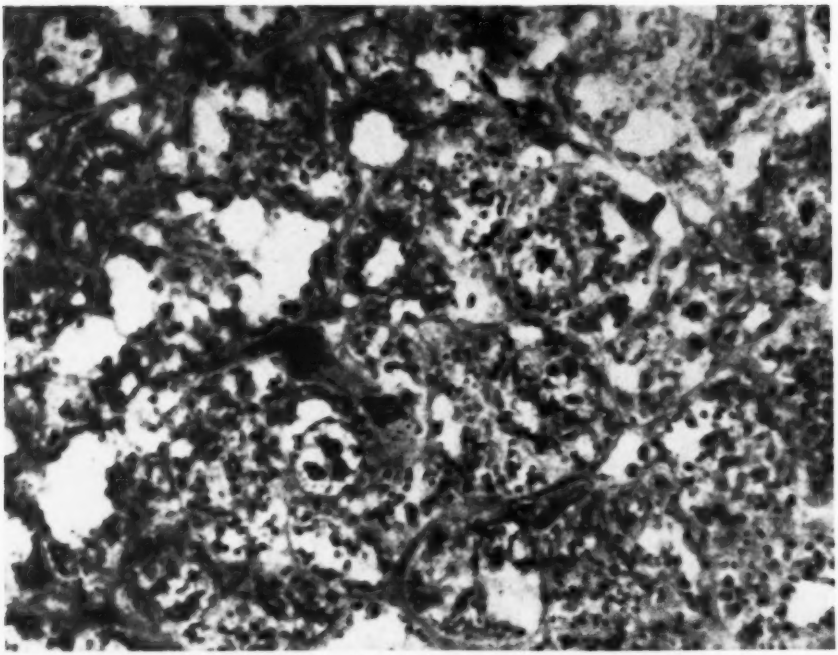

FIGURE 4.—Spherical mitochondria in primary spontaneous hepatoma.

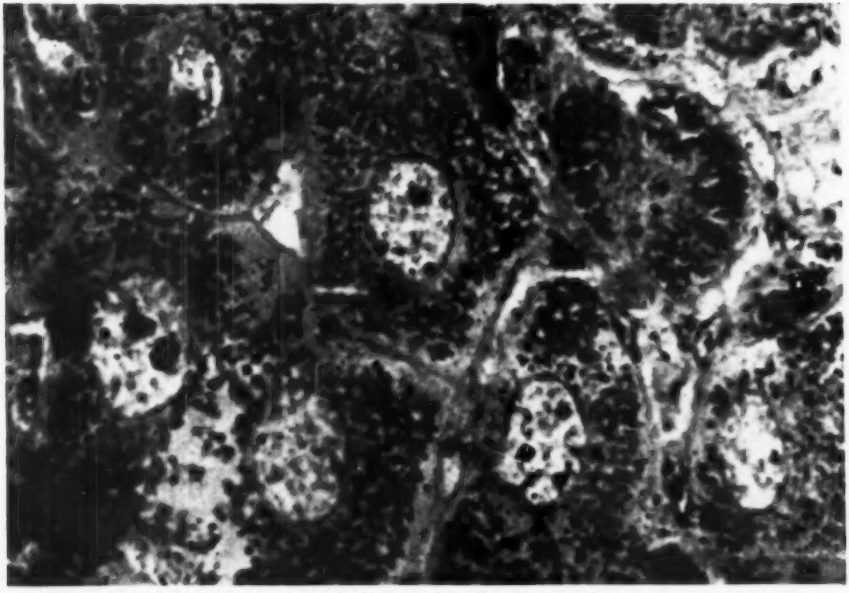

FIGURE 5.—Spherical mitochondria in hepatic cells after *o*-aminoazotoluene treatment.

with *o*-aminoazotoluene. The difference in size and shape of the mitochondria in this case is obvious since the normal hepatic cells are those located near the periphery of a lobule. The mitochondrial type of all 7 spontaneous hepatomas studied was regularly that of a medium-sized sphere. Rarely, plump rods rather than spheres were encountered near the junction with normal liver in primary lesions. They were not found in transplants. Figure 4 demonstrates the typical mitochondrial pattern seen in primary spontaneous hepatomas. In these lesions the mitochondria appeared to be mostly medium-sized spheres. They predominated also in all transplants from these primary tumors.

Mitochondria in regenerating liver showed the normal variation in pattern although the lobular structure was naturally considerably altered. In some cases in which considerable necrosis occurred, the mitochondria in the majority of the remaining hepatic cells appeared as spheres or short rods (fig. 5). The ground glass cells which appeared after CCl_4 treatment usually contained relatively few lightly staining filamentous mitochondria. Frequently scattered among the ground glass cells were other cells undergoing degeneration as evidenced by their few, large, spherical mitochondria.

The Golgi Apparatus

The Golgi apparatus of normal liver cells regularly showed both a juxtanuclear and a peripheral distribution. The amount of Golgi material in these two intracellular zones varied considerably, but a study of serial sections revealed that the two regions were present in all cells studied. The juxtanuclear portion was concentrated on the side toward the bile capillary, and the peripheral portion showed a condensation in intimate contact with the bile capillary itself (fig. 6). The Golgi apparatus of regenerating hepatic cells, following CCl_4 treatment or partial hepatectomy as well as after bile duct ligation, was essentially normal in structure. In partial hepatectomy some hypertrophy of the Golgi material occurred, but its distribution remained normal (fig. 7). After bile duct ligation there was no hypertrophy, and the Golgi material showed normal distribution (fig. 8). The Golgi apparatus of ground glass cells found near the central vein in hepatic lobules of CCl_4-treated specimens was normal in distribution but appeared to be somewhat reduced in amount (fig. 9). Frequently cells were found in CCl_4-treated livers which contained large osmiophilic droplets. These cells were present in the same areas as the ground glass cells, and they often contained material probably representing a disintegrating Golgi apparatus existing as dispersed osmiophilic granules. In other such cells no trace of Golgi material could be identified.

The pattern of the Golgi apparatus in all of the hepatomas was strikingly constant. It varied in detail but appeared without exception as a well-developed juxtanuclear network. No case of peripherally distributed Golgi apparatus has as yet been encountered. Figure 10 shows the Golgi apparatus found regularly in CCl_4-induced hepatomas. This figure shows that when the Golgi material approaches the cell periphery it is due to the direct extension of the hypertrophied juxtanuclear network. No separate peripheral Golgi network is evident. A similar pattern occurred in *o*-aminoazotoluene-induced hepatomas (fig. 11).

Satisfactory demonstration of the Golgi apparatus in spontaneous hepatomas was usually rendered rather difficult by the large amounts of osmiophilic material,

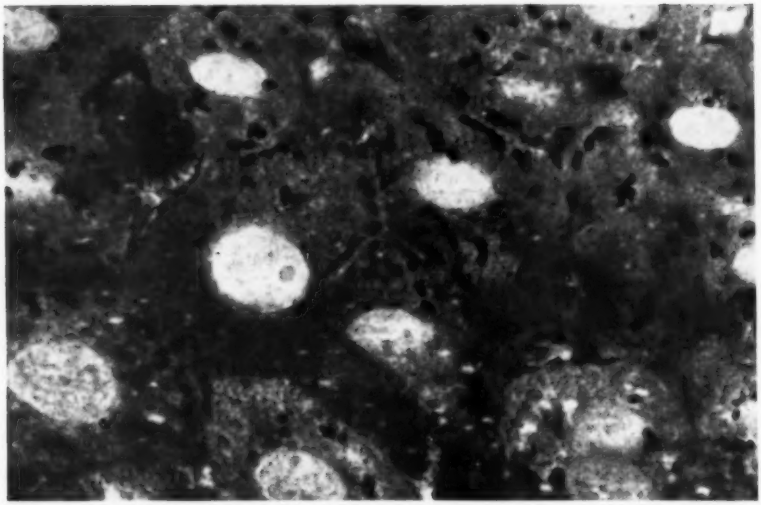

FIGURE 6.—Golgi apparatus in normal liver. Note partly peripheral and partly juxtanuclear position of the Golgi material.

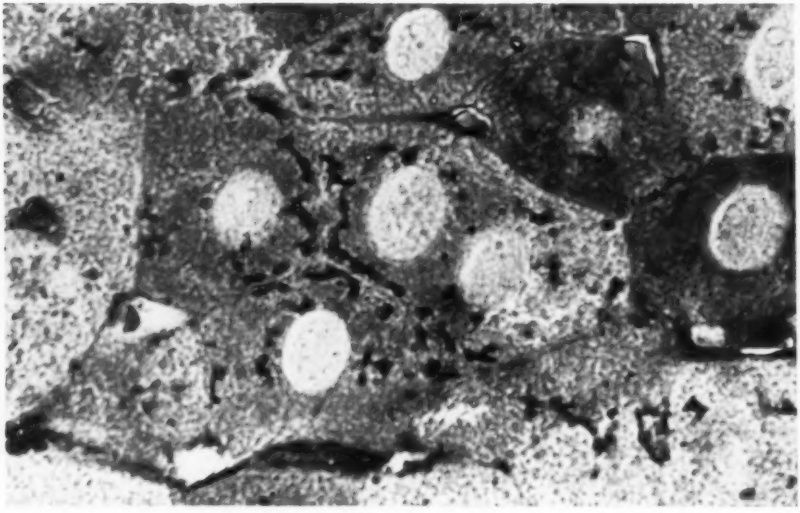

FIGURE 7.—Golgi apparatus in hepatic cells after partial hepatectomy. The distribution of the apparatus is essentially normal though somewhat hypertrophied.

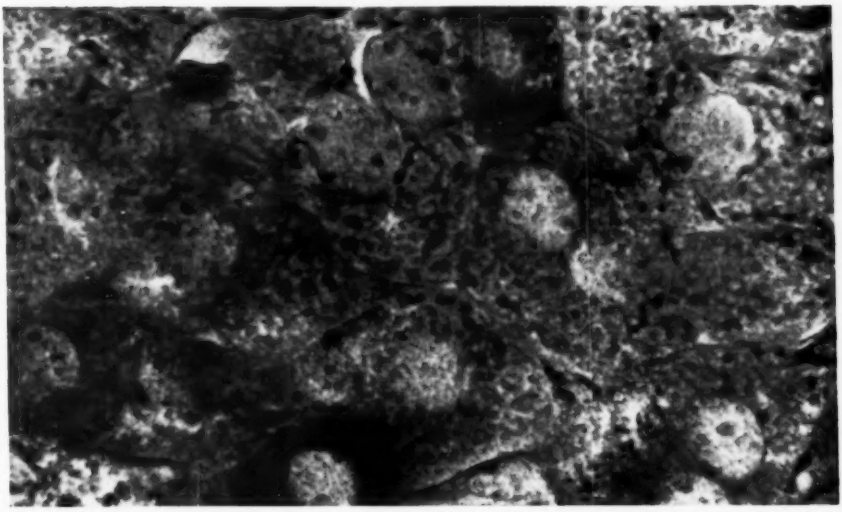

FIGURE 8.—Golgi apparatus in hepatic cells 8 days after bile duct ligation. Normal distribution.

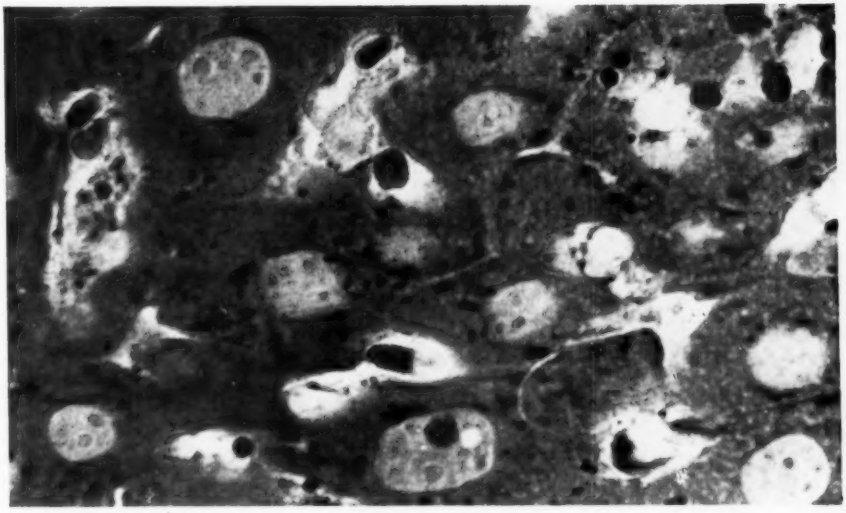

FIGURE 9.—Golgi apparatus of ground glass hepatic cells after CCl_4 treatment in an area free of tumor.

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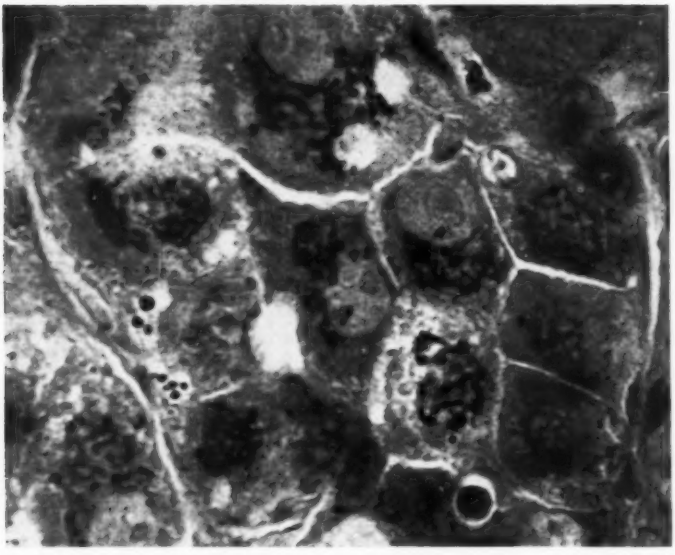

FIGURE 10.—CCl_4-induced hepatoma. Note rather large juxtanuclear mass of Golgi material.

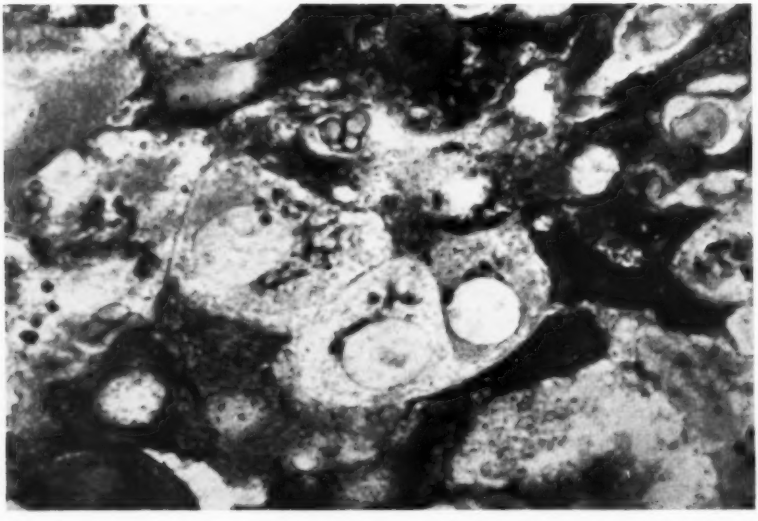

FIGURE 11.—From a section of a transplant of an *o*-aminoazotoluene-induced hepatoma showing juxtanuclear position of the Golgi apparatus.

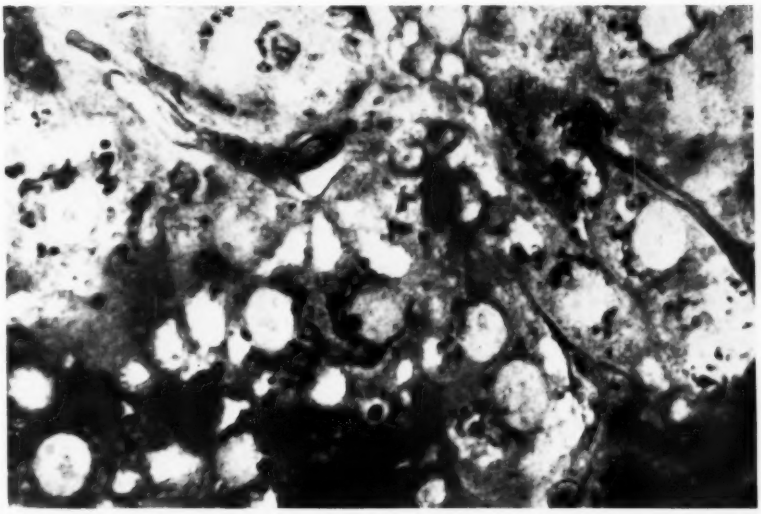

FIGURE 12.—Primary spontaneous hepatoma showing juxtanuclear Golgi apparatus.

probably lipoidal in nature, characteristic of these lesions. Figure 12 shows the Golgi apparatus in a transplant of a spontaneous hepatoma in an area relatively free of lipoid. The structure of the Golgi apparatus has the same general pattern as that found in the induced neoplasms.

DISCUSSION

There are two main points which must be considered in connection with the above observations: (1) The question of the neoplastic nature of these growths; and (2) the significance and diagnostic value of the changes in the mitochondria and Golgi apparatus in mouse hepatomas.

With regard to the first of these questions, these growths are considered to be neoplastic, and evidence in support of this has been described (Edwards (*4*), Edwards, Dalton, and Andervont (*6*), Burns and Schenken (*7*) and Strong and Smith (*8*)). The cells of both induced and spontaneous growths have been shown to possess mitochondrial patterns of a type which make them readily distinguishable from normal liver cells. These growths differ from regenerating hepatic cells which possess normal mitochondrial and normal Golgi patterns. Furthermore, it has been shown with regard to tumors that these intracellular structures maintain their characteristic patterns in transplants. While glycogen was often present in considerable amounts in these growths, there was no evidence of bile secretion in any of them. The evidence suggests that while these cells were superficially much like normal hepatic cells, they were in fact neoplastic.

In regard to the second point, the marked changes in the structure and location of the Golgi apparatus in hepatoma cells are of considerable significance. Cramer and Ludford (*9*) described a cyclic change in the Golgi apparatus of hepatic cells under experimental conditions. They suggested that an exocrine secretory cycle exists in the liver cell as it does in other epithelial-cell types. They further suggested that this cyclic secretion of bile is reflected in the variations in structure and position of the Golgi apparatus which they observed.

The resting cell was considered to be characterized by the possession of a condensed Golgi network in a juxtanuclear position while the actively secreting cell was presumed to possess a dispersed Golgi apparatus in a peripheral position concentrated near the bile capillaries. Such a secretory cycle in normal cells quite conceivably does exist, and the considerable variation in the amount of peripheral and juxtanuclear Golgi material observed in normal hepatic cells supports such a concept. However, in the observations made on numerous sections of normal liver cells no single case has been found in which the Golgi material was completely peripheral or completely juxtanuclear. It is probable that new Golgi material is formed near the nucleus before the peripherally located material is entirely lost. Following the interpretation of Cramer and Ludford (*9*), the characteristic juxtanuclear position of the Golgi apparatus in all of the hepatomas so far studied would suggest that these hepatoma cells are in a resting state as far as exocrine secretory activity is concerned. This concept is supported by the absence of any evidence of bile secretion.

The presence of filamentous mitochondria in induced hepatoma and of spherical ones in spontaneous hepatoma is of interest. Many attempts have been made to correlate changes in mitochondrial form with changes in cellular activity. Observations on amphibian (Noll (*10*)) and chick (Dalton (*11*)) hepatic cells suggest that spherical forms are correlated with low metabolic activity, filamentous forms with heightened activity. The work of Noël (*5*) on the mouse liver, however, indicates that spherical mitochondria are present in the zone of permanent activity near the periphery of the lobule while filamentous mitochondria are characteristic of cells in the zone of permanent repose near the central vein. This suggests that filamentous mitochondria are indicative of decreased activity in the mouse hepatic cell. In the present work filamentous mitochondria are present in the more rapidly growing induced hepatomas and spherical mitochondria in the slower growing spontaneous hepatomas. It is argued by many that whether tumors arise spontaneously or are induced, the fundamental process of neoplasia is the same. In the mouse hepatomas, obviously derived from the same parent cell the mitochondrial type is dependent upon the nature of origin of the neoplasm, whether induced or spontaneous.

The use of the mitochondria and the Golgi apparatus as diagnostic criteria for the classification of liver tumors seems to be possible. However, its value appears to depend partly on the unusual and highly developed Golgi apparatus of normal hepatic cells and partly on the constant variation in mitochondrial form present in the normal hepatic lobule. Similar changes in the mitochondria and the Golgi apparatus have been noted in a transplantable hepatoma of the rat (*12*). Further studies on human tumors are now in progress.

SUMMARY

The mitochondria and Golgi apparatus of induced and spontaneous hepatoma cells are compared with those of normal hepatic cells and with those of livers after partial hepatectomy, bile duct ligation, and CCl_4 treatment.

Mitochondria of cells of induced hepatoma are regularly fine filaments while those of the spontaneous hepatoma are spherical.

The mitochondrial type characteristic of a primary hepatoma is also characteristic of the tumor in transplant.

The Golgi apparatus of hepatoma cells is in the form of a variably condensed juxtanuclear network.

It is suggested that some relationship ex-

ists between the presence of this type of Golgi apparatus and the absence of exocrine secretory activity in the hepatoma cell.

The diagnostic value of the mitochondria and the Golgi apparatus with respect to neoplasms of the mouse liver is discussed.

REFERENCES

(*1*) COWDRY, E. V.: Properties of cancer cells. Arch. Path., 30: 1245–1274 (1940).

(*2*) BAGOZZI, U. C.: Studi sulla fine struttura della cellule neoplastiche. Nota I. L'apparato reticulare interno. Tumori, 7: 266 (1933).

(*3*) ———: Studi sulla fine struttura della cellule neoplastiche. Nota II. L'apparata mitochondriale. Tumori, 7: 280 (1933).

(*4*) EDWARDS, J. E.: Hepatomas in mice induced with carbon tetrachloride. J. Nat. Cancer Inst., 2: 197–199 (1941).

(*5*) NOËL, R.: Recherches histopathololgiques sur la cellule hepatique des mammifères. Arch. d'anat. micr., 19: 1–158 (1923).

(*6*) EDWARDS, J. E., DALTON, A. J., and ANDERVONT, H. B.: Pathology of a spontaneous hepatoma in a C3H mouse. J. Nat. Cancer Inst., 2: 555–563 (1942).

(*7*) BURNS, E. L., and SCHENKEN, J. R.: Spontaneous primary hepatomas in mice of strain C3H. A study of incidence, sex distribution and morbid anatomy. Am. J. Cancer, 39: 25–35 (1940).

(*8*) STRONG, L. C., and SMITH, G. M.: Successful transplantation of a hepatoma in mice. Am. J. Cancer, 28: 112–114 (1936).

(*9*) CRAMER, W., and LUDFORD, R. J.: On the cellular mechanism of bile secretion and its relation to the Golgi apparatus of the liver cell. J. Physiol., 62: 74–80 (1926).

(*10*) NOLL, A.: Über granula und mitochondrien der Leberzellen. Ztschr. f. Zellforsch. u. mikr. Anat., 9: 281–286 (1929).

(*11*) DALTON, A. J.: The ontogenetic history of the mitochondria and Golgi network of the hepatic cells of the chick. Anat. Rec., 58: 321–347 (1934).

(*12*) WHITE, J., DALTON, A. J., and Edwards, J. E.: Pathology of rat hepatoma 31. J. Nat. Cancer Inst., 2: 539–554 (1942).

Influence of Estradiol Benzoate on Epidermal Methylcholanthrene Carcinogenesis[1]

By F. X. PALETTA,[2] *research fellow*, and PAUL F. MAX, *assistant in gynecology, Barnard Free Skin and Cancer Hospital, St. Louis*

Epidermal methylcholanthrene carcinogenesis has been under intensive study at the Barnard Hospital. Since clinical dosages of the estrogens have been ever increasing, it seemed worth while to test the influence of estradiol benzoate on this type of cancer formation.

The literature shows that the estrogens have proven important etiological factors in the experimental production of mammary-gland carcinoma (*1–3*) and of carcinoma of the cervix (*4–6*). Burrows (*7*) and Cramer and Horning (*8*) painted the skins of mice with estrogenic hormones for long periods without producing carcinoma. Schockaert (*9*), using weekly injections of 20 I. U. of theelin in oil, observed that mice treated with both tar and folliculin developed carcinomas of the skin earlier than did mice in the control series and that the carcinomas grew more rapidly. Gilmour (*10*) found that carcinomas inoculated into estrogen-painted mice grew no more rapidly than in mice in the group receiving no estrogen. She did, however, find a marked acceleration in development and growth of carcinomas in estrogen-treated mice that were painted with a carcinogenic agent (benzpyrene). On the other hand, Perry and Ginzton (*11*) reported that the incidence of carcinoma of the skin in female mice induced by 1, 2, 5, 6-dibenzanthracene was not intrinsically affected by either spaying or the use of theelin.

Methylcholanthrene has proved to be one of the most potent of the cancer-producing hydrocarbons (*12, 13*) and was the agent used in the experiment reported herein. It has been definitely established by numerous workers that the molecular structure of cancer-producing hydrocarbons is closely related to naturally occurring products (sterols, bile acids, ovarian hormones, vitamin D, cardiac poisons, male hormones, and certain of the vegetable alkaloids). These workers have pointed out the possibility that the molecular structures of the sterols and bile acids may at times be transformed by some metabolic process into carcinogenic agents. At the present there is no evidence that this actually occurs. Wieland and Dane (*14*), however, were successful in isolating methylcholanthrene as a degradation product from desoxycholic acid, one of the principal bile acids.

The purpose of the present experiment was to see what influence the keeping of mice constantly in estrus would have on methylcholanthrene skin carcinogenesis.

MATERIALS AND METHODS

Virgin female mice 3 months old, of inbred Swiss stock, were divided into 2 groups of 40 each. In the first group, the hair over the abdomen was cut short with scissors, and 3 drops of α-estradiol benzoate in the form of a 0.01-percent solution in chloroform was painted on the abdominal

[1] *Aided by a grant from the National Cancer Institute.*

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skin every 4 days. The calculated dosage of estrogen received by each mouse at one application was 37.5 rat units. Eighteen hours after each estrogen application, a 0.3-percent solution of methylcholanthrene in benzene was painted on an area of skin about 5 mm. in diameter at the interscapular region. A No. 5 camel's-hair brush was used in applying the compounds.

Laqueur (*15*) reported that 0.1 microgram of estrone was required to produce estrus in two-thirds of the mice he injected. According to our calculations of the estrogen we used, expressed in estrone equivalent, 7.3 rat units of α-estradiol benzoate are equivalent to 1 microgram of estrone. Vaginal smears taken daily for 4 days on five virgin female mice, which had received the amount of estrogen in one painting in our experiment, showed many cornified cells, indicating that the mice were continually in estrus. Distention of the urinary bladder was frequently present in most of the mice, and the bladder was decompressed manually.

The second group of mice were the control series. They were painted with 0.3 percent methylcholanthrene in benzene at the same times as the first group. No estrogens were applied to the control group. All the mice were weighed once a week, and a record was kept of the time at which the skin lesions appeared. When a tumor was thought to be malignant, biopsies were taken and microscopic sections made. The criterion used for diagnosis of squamous-cell carcinoma was the presence of neoplastic cells invading muscles. At the close of the experiment all the mice were killed. All breasts were examined grossly for signs of carcinoma. In addition, the breasts, vaginae, cervices, and uteri of mice from each group were sectioned and studied microscopically.

RESULTS

The results of the experiments are shown graphically in figure 1.

The duration of treatment is plotted horizontally at weekly intervals; the percentages of the total number of mice having tumors are shown vertically. Each weekly recording comprises two columns, the one to the left represents the control series and the one to the right the estrogen-treated series. The highest point on each column represents the percentage of the 40 mice in each series having tumors. The bottom of each column shaded in black shows the percentage of the 40 mice in each

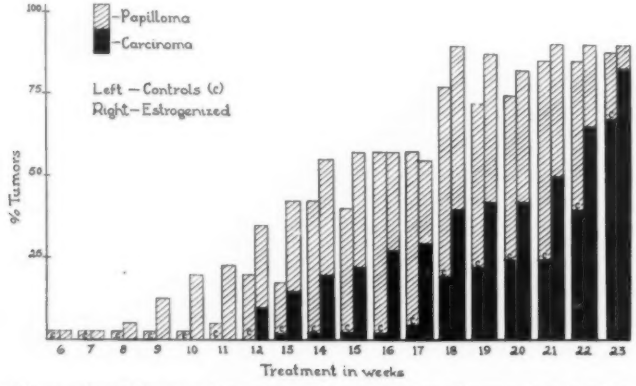

FIGURE 1.—Incidence of papilloma and carcinoma in two groups of virgin female mice of inbred Swiss stock, one group being treated with estrogen, and the other group serving as controls.

series that have malignant tumors. The percentage of the 40 mice having benign tumors is indicated by the upper part of the column which is cross-hatched.

In both the control and the estrogen-treated series, the first papilloma appeared in the sixth week. Ten weeks after the beginning of the experiment 20 percent of the estrogen-treated mice had benign tumors, whereas only 2.5 percent of the controls had developed this type of tumor. The first squamous-cell carcinoma occurred in the estrogen-treated series at the twelfth week, at which time four in the same series had malignant tumors. It is interesting to compare the results at the sixteenth and at the twenty-first week. At these particular intervals, the total percentage of tumors, both benign and malignant, was about the same, being slightly greater in the estrogen-treated mice in the twenty-first week. However, a comparison of the mice at these intervals showed that 27.5 percent of the estrogen-treated animals and 2.5 percent of the controls had developed malignant tumors at the sixteenth week, and 50 and 25 percent, respectively, at the twenty-first week. At the twenty-third week, 82.5 percent of the mice in the estrogen-treated group had malignant tumors, as compared with 67.5 percent of those in the control group. In the estrogen-treated group two mice did not develop tumors and one died, while in the control group four mice did not develop tumors and one died.

A record was kept of the time at which the benign tumors appeared, and particular attention was paid to the interval required for each benign tumor to undergo malignant changes. The results are shown in table 1. The first column gives the time interval required for benign tumors to become malignant. Zero indicates that the tumors were rapidly growing carcinomas from the beginning. The second column shows the number of mice in the estrogen-treated group having benign tumors that showed malignant changes at the various intervals, and in the third column are given the results in the control group.

TABLE 1.—*Time that elapsed before benign tumors became malignant*

Time required for malignant transformaton (weeks)	Mice developing carcinomas	
	Estrogenized	Controls
	Number	*Number*
0	1	1
1	5	0
2	3	3
3	10	2
4	5	3
5	4	4
6	1	6
7	2	3
8	0	3
9	1	0
10	1	1
11	0	1
Mean	3.9	5.4
Standard deviation	2.23	2.46
Standard error	.154	.212
Difference	1.8±2.96; P<0.01	

The means of the time required for the estrogenized and control mice to develop malignant tumors are given (below table 1), with their standard deviation and standard error as obtained by the use of the method of weighted means. A contrast of these differences shows that the control animals took 1.8 weeks longer to develop malignancy than did the estrogenized animals. When this difference is analyzed by contrast of the standard errors, it is significant, since a probability of occurrence is less than 0.01.

No significant differences were noted when the rate of growth in the tumors appearing early in the experiment in both series was plotted. A record of the weights of the mice taken at weekly intervals showed, at the end of the experiment, an average increase of 2.4 gm. per mouse in the estrogen-treated group and a greater increase, 4.7 gm., in the control group.

None of the mice in the estrogen-treated

series showed any evidence of carcinoma of the breast, although the breasts of these animals were more prominent than were those of the controls. Sections of the breasts showed marked hypertrophy of the glandular elements but failed to show any adenocarcinoma. The vaginae, cervices, and uteri also showed the expected result of hypertrophy and proliferation, but no carcinomas were found.

DISCUSSION

The results indicate that in Swiss mice the application of α-estradiol benzoate accelerates the transformation of benign tumors into malignant tumors and shortens the induction period in methylcholanthrene skin carcinogenesis. Gilmour (*10*), in studying the influence of estrogens on the carcinogenic action of 3,4-benzpyrene, reported that the average time for papillomas to show malignant characteristics was only 3 days less in the estrogen-treated group than in the control group. We found the difference to be 1.8 weeks less in the estrogen series than in the controls. However, we attempted to keep the mice constantly in estrus and used α-estradiol benzoate, a more potent estrogenic compound and effective for longer periods, whereas Gilmour used ketohydroxyestrone.

Such results are interesting since they show the influence that estrogens have on the carcinogenic action of the closely related hydrocarbon, methylcholanthrene, which is the only hydrocarbon of demonstrated carcinogenic activity that has been obtained from substances normally present in the body.

Whether the action of the estrogens is a general systemic stimulation resulting in increased proliferative activity of the epidermal cells or represents a synergistic action on methylcholanthrene activity is difficult to say. Loeb and Haven (*16*) reported that there is a slight increase in epidermal proliferative activity following the injection of follicular extract into hysterectomized guinea pigs. Estrogens do have a selective proliferative action on the epithelial cells of the vagina and cervix (Allen (*17*, *18*)). In order for one to interpret the nature of the epidermal response to the influence of estrogens in methylcholanthrene skin carcinogenesis, further work will be necessary.

SUMMARY

The application of α-estradiol benzoate accelerated the transformation of benign tumors into malignant tumors in methylcholanthrene skin carcinogenesis in virgin female mice of a Swiss inbred stock.

Estrogens shortened the induction period for the appearance of carcinomas in the estrogenized mice as compared with the controls.

REFERENCES

(*1*) Lacassagne, A.: Apparition de cancers de la mamelle chez la souris mâle, soumise à des injections de folliculine. Compt. rend. Acad. d. sc., 195: 630–632 (1932).

(*2*) ———: Influence d'un facteur familial dans la production, par la folliculine, de cancers mammaires chez la souris mâle. Compt. rend. Soc. de biol., 114: 427–429 (1933).

(*3*) ———: Hormonal pathogenesis of adenocarcinoma of breast. Am. J. Cancer, 27: 217–228 (1936).

(*4*) ———: Modifications progressives de l'utérus de la souris sous l'action prolongée de l'oestrone. Compt. rend. Soc. de biol., 120: 1156–1158 (1935).

(*5*) Loeb, L., Burns, E. L., Suntzeff, V., and Moskop, M.: Carcinoma-like proliferations in vagina, cervix, and uterus of mouse treated with estrogenic hormones. Proc. Soc. Exper. Biol. & Med., 35: 320–322 (1936).

(*6*) Gardner, W. U.: Influence of estrogenic hormones on abnormal growths. Some fundamental aspects of the cancer problem. Cancer Probl., Symposium, pp. 67–75 (1937).

(7) BURROWS, H.: Pathological changes induced in mamma by oestrogenic compounds (Hunterian lecture). Brit. J. Surg., 23: 191–213 (1935).

(8) CRAMER, W., and HORNING, E. S.: Experimental production of oestrin of pituitary tumours with hypopituitarism and of mammary cancer. Lancet, 1: 247–249 (1936).

(9) SCHOCKAERT, J. A.: Grossesse, cancer et folliculine. Bruxelles-méd., 15: 1010–1012 (1935).

(10) GILMOUR, M. D.: Investigation into influence of oestrone on growth and genesis of malignant cells. J. Path. & Bact., 45: 179–188 (1937).

(11) PERRY, I., and GINZTON, L.: Development of tumors in female mice treated with 1:2:5:6-dibenzanthracene and theelin. Am. J. Cancer, 29: 680–704 (1937).

(12) FIESER, L. F., FIESER, M., HERSHBERG, E. B., NEWMAN, M. S., SELIGMAN, A. M., and SHEAR, M. J.: Carcinogenic activity of cholanthrenes and of other 1:2-benzanthracene derivatives. Am. J. Cancer, 29: 260–268 (1937).

(13) FIESER, L. F., and SELIGMAN, A. M.: The synthesis of methylcholanthrene. J. Am. Chem. Soc., 57: 228–229 (1935).

(14) WIELAND, H., and DANE, E.: Untersuchungen über die Konstitution der Gallensäuren. LII. Mitteilung. Über die Haftstelle der Seitenkette. Ztschr. f. physiol. Chem., 219:240–244 (1933).

(15) LAQUEUR, E.: Zur Eichung brunstgebender Stoffe. Ein Wort über therapeutischen Wirkungswert. Klin. Wchnschr., 14:339–341 (1935).

(16) LOEB, L., and HAVEN, F.: Relation between functional states of sex organs in female guinea-pig and cell proliferation in epidermis. Anat. Rec., 43: 1–26 (1929).

(17) ALLEN, E.: Menstrual cycle of monkey, *Macacus rhesus:* Observations on normal animals, effects of removal of ovaries and effects of injections of ovarian and placental extracts into spayed animals. Contrib. Embryol. (No. 98), 19: 1–44 (1927).

(18) ————: Further experiments with ovarian hormone in ovariectomized adult monkey, *Macacus rhesus*, especially degenerative phase of experimental menstrual cycle. Am. J. Anat., 42: 467–487 (1928).

Some Observations on Atrophic Gastritis and Gastric Cancer[1,2]

By NATHAN SHAPIRO,[3] *instructor in medicine*, LEON SCHIFF, *associate professor of medicine*, *University of Cincinnati Medical School*, MARY M. MAHER, *fellow in gastroenterology*, *Cincinnati General Hospital*, and MAX M. ZINNINGER, *associate professor of surgery*, *University of Cincinnati Medical School*

INTRODUCTION

The correlation of clinical and anatomical material has demonstrated the frequent association of atrophic gastritis and gastric cancer (*1–5*). Konjetzny (*2*) and Hurst (*3*) have expressed the belief that atrophic gastritis is a forerunner of gastric cancer. Konjetzny has adduced histologic evidence showing transitional stages from chronic atrophic gastritis through polyposis to gastric cancer. Rhoads (*6*) has stated:

> The association of pernicious anemia, in which gastritis is always present, with gastric cancer appears to be more frequent than could be explained by the laws of chance * * * (*6, p. 517*).

The invention of the Wolf-Schindler flexible gastroscope in 1932 has given considerable impetus to the clinical study of gastric disease, particularly chronic gastritis. Since 1936 we have performed satisfactory gastroscopic examinations on 124 patients with chronic atrophic gastritis and 50 of 60 patients with gastric cancer proved either by operation or autopsy. In this laboratory the attempt is being made to throw some light on the relationship, if any, of gastritis and gastric cancer by repeatedly examining patients with atrophic gastritis in order to detect the early development of gastric cancer. In addition, patients with gastric cancer are being studied, with particular emphasis on the presence or absence of atrophic gastritis. Some preliminary observations in connection with the latter phase of the problem are reported in this paper.

In 35 of our cases of gastric cancer a sufficiently large section of well-preserved gastric mucosa is available for correlation (of mucosal changes) with clinical and gastroscopic observations.[4,5] Satisfactory gastroscopic examinations were carried out in 28 of these 35.

Since October 1939 all stomach specimens have been cut into large blocks, and slides have been made which are capable of holding a section of one-third to one-half of the stomach. These large sections contain both the lesser and the greater curvature of the stomach and thereby provide an excellently preserved mucosal surface which may be followed from the pylorus to the cardia. The point of junction of mucosa and tumor is readily seen. The mucosa may accordingly be examined at points far removed from the tumor. One can then evaluate the degree and extent of the gastritis both in the vicinity of the tumor and elsewhere (fig. 1).

[1] *This work was aided by a grant from the National Cancer Institute.*

[2] *From the Departments of Internal Medicine and Surgery of the University of Cincinnati Medical School, and the Gastric Research Laboratory of the Cincinnati General Hospital.*

[3] *Trainee in diagnosis and treatment, National Cancer Institute, 1940–42.*

[4] *The remaining 25 cases were eliminated on grounds such as inadequate size of mucosa included in operative biopsy, post mortem change, or failure to obtain more than a lymph node biopsy.*

[5] *In the cases of patients that came to autopsy, the stomach was usually injected immediately after death (always within an hour) with 500 cc. of 10 percent formalin through the abdominal wall according to the method of Faber and Bloch* (7). *More recently we have been passing an Ewald stomach tube immediately post mortem and instilling the 10 percent formalin in this manner. At operation the specimen was obtained by one of us immediately after its removal from the body. It was gently stuffed with gauze soaked in 10 percent formalin and then immersed in its entirety in 10 percent formalin.*

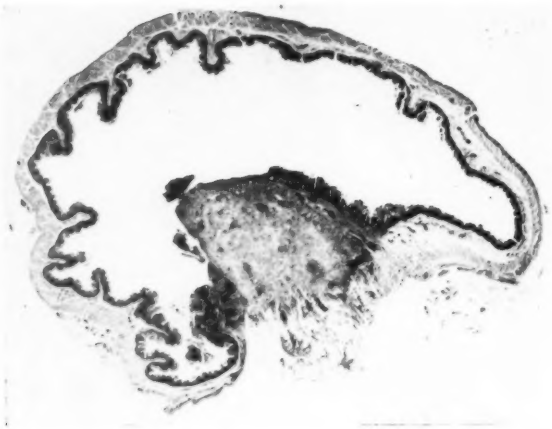

FIGURE 1.—Large section of stomach showing a carcinoma along the lesser curvature.

GASTROSCOPIC CRITERIA OF ATROPHIC GASTRITIS

The gastroscopic criteria of atrophic gastritis which have been used are the presence of grayish or greenish-gray patches in the mucosa or the demonstration of either reddish or bluish branching blood vessels. We have not relied on the absence of rugae or the presence of improminent rugae as evidence of atrophic gastritis, because such changes may depend upon the degree of insufflation at the time of the examination.

PATHOLOGIC CRITERIA OF ATROPHIC GASTRITIS

The pathologic criteria of atrophic gastritis which we have followed are:

Atrophic gastritis, plus-minus.—Sparse infiltration of the interstitial tissues, with plasma cells and lymphocytes, with a very slight degree of one or more of the other changes noted below, such as goblet cells, etc. The infiltration must be more than superficial, i. e., it must be found throughout the width of the mucosa.

Atrophic gastritis, grade 1.—Greater infiltration of the interstitial tissues. There should be a mild degree of more than one of the following changes: goblet cells replacing the gastric epithelial cells, cystic dilatation of the glands, decrease in the number of glands, increase in lymph follicles, thinning of the gastric mucosa as a whole, some degree of infiltration of the muscularis mucosa, and edema of the interstitial tissues.

Atrophic gastritis, grade 2.—A slightly greater degree of the above-mentioned changes with an increase in the number of types of change (in grade 1, only one or two of the changes may be present; they should be more fully represented in this category). Slight distortion of the individual gland cells and glands may be present, and the chief and parietal cells may be decreased in number.

Atrophic gastritis, grade 3.—Moderately advanced degree of most of the above-mentioned changes should be present. It should be noted that the change of one type may be much more advanced than of another, for example, the glands may be markedly decreased and the mucosa thinned, yet the goblet cells may not be frequent. Distortion of cells and glands may or may not be present.

Atrophic gastritis, grade 4.—The mucosa is quite thin (one-fourth of its usual width or less). The glands are almost gone. Goblet cells are profuse; the interstitial tissues are infiltrated and contain excess connective tissue; chief and parietal cells are sparse to absent; the gastric pits are short and wide; cystic dilatation is present in varying numbers; cellular and glandular elements may show distortion of conformation. The picture is very striking and usually easier to grade than are the intermediate types.

Atrophic gastritis of one grade or another was found to be present micro-

scopically in 28 of the 35 cases above referred to (p. 583). Schindler found gastritis, usually of the atrophic type, in 33 of 49 patients with gastric cancer (*6, pp. 521–522*).

In the 28 cases of gastric cancer in which satisfactory gastroscopy was carried out and in which adequate sections are available, we found (1) that gastritis was present, both gastroscopically and microscopically, in 10 instances, (2) that gastritis was absent, both gastroscopically and microscopically, in 7, and (3) that gastritis was not observed gastroscopically, but was present microscopically in 11 (1, plus-minus; 1, one-plus; 3, two-plus; 5, three-plus; and 1, four-plus).

In 4 of the 11 cases in which the gastritis was overlooked gastroscopically, the rugae were improminent or absent. In 2, the tumor was extensive, and there was a heavy collection of necrotic debris which probably concealed the gastritis.[6] In one, the tumor itself was not seen and the gastritis was localized microscopically to its vicinity. In another, the presence of superficial gastritis may have masked the associated atrophic gastritis. In the remaining 3 cases, there was no explanation for the discrepancy between the gastroscopic and microscopic changes. In view of the fact that gastritis may not be detected gastroscopically in the presence of gastric cancer,[7] this report, as it concerns gastritis, is limited to only those cases having adequate microscopic sections.

RELATIONSHIP OF DURATION OF SYMPTOMS TO PRESENCE OF ATROPHIC GASTRITIS IN PATIENTS WITH GASTRIC CANCER

Usland (*8*) and Kapp (*9*) considered the long duration of digestive symptoms prior to the clinical diagnosis of gastric cancer as an argument in favor of the pre-existence of gastritis. Usland found that 26 of 94 patients who had been operated upon for cancer of the stomach had suffered from dyspeptic symptoms more than 5 years prior to the clinical manifestations of cancer, and 18 of 120 patients who had suffered from symptoms of chronic gastritis for at least 2 years when first seen later developed gastric carcinoma. Kapp found that symptoms, presumably gastritic, were present at least 5 years before the diagnosis of gastric cancer was made in 120 persons. In another series, 21 of 157 persons with a clinical diagnosis of gastritis developed gastric cancer in 5 years or more after the diagnosis of gastritis was made.

Table 1 presents an analysis of the histories of 34 of the 35 patients with proven gastric cancer,[8] with an attempt to evaluate the duration of digestive symptoms as related to atrophic gastritis. The table shows that gastritis was present in 13 of the 19 patients with digestive disturbances of 8 months' duration or less, whereas it was present in 14 of the 15 patients reporting digestive distress in the periods from 9–12 months to life. This observation would

TABLE 1.—*Relationship of duration of symptoms to presence of atrophic gastritis in gastric carcinoma*

Duration of symptoms	Cases without gastritis	Cases with gastritis	Total cases
	Number	*Number*	*Number*
4 months or less	5	8	13
5–8 months	1	5	6
9–12 months	0	3	3
1–2 years	1	1	2
2–3 years	0	3	3
3–4 years	0	1	1
4–5 years	0	1	1
5–6 years	0	1	1
6–7 years	0	0	0
7–8 years	0	0	0
8–9 years	0	1	1
10 years	0	1	1
13 years	0	1	1
"Life"	0	1	1
Total	7	27	34

[6] *Carey* (10) *was unable to observe satisfactorily the gastric mucosa in 20 of 39 cases of gastric cancer.*

[7] *This also explains our contradiction of previous inferences which were based primarily upon gastroscopic appearances* (6, pp. 519–521).

[8] *In one case the patient was unable to date the onset of symptoms.*

tend to support the experience of Usland and Kapp.

GASTRIC CARCINOMA AND GASTRIC ACIDITY

The frequency of achlorhydria in gastric cancer is well-established. Ask-Upmark (*11*) noted a significant difference between growths involving the pyloric region in which 40 percent showed free hydrochloric acid, and growths restricted to the body of the stomach in which 4 percent had free hydrochloric acid. Polland and Bloomfield (*12*) found that all patients with free acid had lesions at the pylorus, but that anacidity occurred with growths located in any part of the stomach. Figure 2 shows that in our experience there is no correlation between the gastric acidity and the site of the cancer. These observations are in agreement with those of Comfort and Vanzant (*13*), Hartman (*14*), and Brunschwig and his coworkers (*15*).

GASTRITIS AND THE LOCATION OF GASTRIC CANCER

In 28 cases in which associated atrophic gastritis was found, the gastric cancer was located in the prepyloric region or antrum or in both situations in 12, in the pars media in 8, and was diffusely infiltrating in character in 8.

RELATIONSHIP OF ACIDITY TO ATROPHIC GASTRITIS IN GASTRIC CANCER

The following tabulation strongly suggests that achlorhydria occurs more frequently in the cases of gastric cancer associated with gastritis than in those that are not. On the other hand, achlorhydria (in gastric cancer) may occur in the absence of gastritis as would be expected from experiences with achlorhydria in general and from the work of Brunschwig (*15*) and others (*6, pp. 519–521; 10; 16*). The five patients that had no gastric analysis are not included in the tabulation.

FIGURE 2.—Gastric acidity and location of gastric cancer (52 cases).

	No gastritis	*Gastritis*
No free HCl	2	17
Free HCl	4	7

RELATIONSHIP OF ANEMIA TO ATROPHIC GASTRITIS IN GASTRIC CANCER

As the following tabulation shows, anemia is far more common when gastritis is associated with gastric cancer than when it is not.

	No gastritis	*Gastritis*
No anemia [1]	5	10
Anemia	1	18

[1] Red blood cell count, 4.0 million or more. One patient had no blood count and is therefore not included in the tabulation.

On the other hand, there may be no anemia in the presence of gastritis, as is to be expected from the frequent absence of anemia in gastritis that is not associated with gastric cancer [9] (*17, 18*).

RELATIONSHIP OF ANEMIA AND GASTRIC ACIDITY IN GASTRIC CANCER

Figure 3 demonstrates that anemia is much more likely to occur when achlorhydria is associated with gastric cancer than when free acid is present in the gastric contents. This is to be expected in view of the general relationship of achlorhydria and anemia. Comfort and Vanzant (*13*), however, were unable to demonstrate any

[9] *Schiff, L.: Unpublished data.*

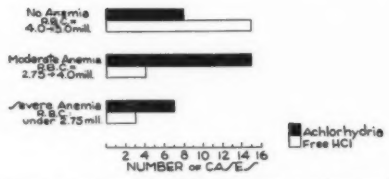

FIGURE 3.—Anemia and gastric acidity in gastric cancer (52 cases).

relationship between the percentage of hemoglobin concentration and the incidence of achlorhydria in cases of gastric cancer.

ANEMIA AND THE LOCATION OF GASTRIC CANCER

According to figure 4, no correlation was demonstrable between the degree of anemia and the location of the cancer. The anemia was in almost all instances hypochromic microcytic or hypochromic normocytic in character.

SUMMARY AND CONCLUSIONS

Thirty-five cases of gastric cancer were selected from a group of 60 proved cases as satisfactory for clinicopathologic study. Microscopic evidence of atrophic gastritis was found in 28 of the 35 (an incidence of 80 percent). Atrophic gastritis was often overlooked gastroscopically in the presence of gastric cancer. Gastritis occurred more frequently in patients having a long history of digestive disturbance. The occurrence of gastritis and the presence or absence of free hydrochloric acid in the gastric contents were not related to the location of the tumor. Achlorhydria and anemia occurred more often in patients with atrophic gastritis.

While it is realized that this study comprises a relatively small series of cases, the frequent coexistence of the two diseases strongly suggests a relationship, the nature of which has yet to be determined.

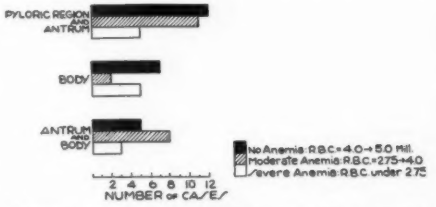

FIGURE 4.—Anemia and the location of gastric cancer (58 cases).

REFERENCES

(1) FABER, K.: Gastritis and its consequences. Oxford Univ. Press, London; Gyldedalske Boghandel Nordisk Forlag, Copenhagen (1935).

(2) KONJETZNY, G. E.: Ueber die Beziehungen der chronischen Gastritis mit ihren Folgeerscheinungen und des chronischen Magenulcus zur Entwicklung des Magenkrebses. Beitr. z. klin. Chir., 85: 455–519 (1913).

(3) HURST, A. F.: Schorstein lecture on precursors of carcinoma of the stomach. Lancet, 2: 1023–1028 (1929).

(4) ORATOR, V.: Beiträge zur Magenpathologie. II. Zur Pathologie und Genese des Carcinoms und Ulcuscarcinoms des Magens. Virchows Arch. f. path. Anat., 256: 202–229 (1925).

(5) SCHINDLER, R.: Gastroscopy: The endoscopic study of gastric pathology. Univ. Chicago Press, Chicago (1937).

(6) RHOADS, C. P.: Gastric cancer as a sequel to gastritis, particularly the gastritis of pernicious anemia. J. Nat. Cancer Inst., 1: 511–522 (1941). Discussion: by Dr. Schiff, pp. 519–521; by Dr. Schindler, pp. 521–522.

(7) FABER, K., and BLOCH, C. E.: Über die pathologischen Veränderungen am Digestionstractus bei der perniciöser Anämie und über die sogenannte Darmatrophie. Ztschr. f. klin. Med., 40: 98–136 (1900).

(8) USLAND, O.: Ueber die Bedeutung der chronischen Gastritis für die Entwicklung des Magenkrebses. Acta chir. Scandinav., 76: 485–499 (1935).

(9) KAPP, H.: Zur Bedeutung der Anamnese des Magenkarcinoms. 2d Congr. internat. de gastro-enterol., Bruxelles, p. 592 (1937).

(10) CAREY, J. B., WETHERBY, M., and YLVISAKER, R. S.: Gastric observations in achlorhydria. Am. J. Digest. Dis., 8: 401–407 (1941).

(11) ASK-UPMARK, E.: On the gastric acidity in cancer of the stomach. Acta chir. Scandinav., 80: 93–102 (1937).

(12) POLLAND, W. S., and BLOOMFIELD, A. L.: Gastric secretion in cancer of the stomach. Bull. Johns Hopkins Hosp., 46: 307–322 (1930).

(*13*) COMFORT, M. W., and VANZANT, F. R.: Gastric acidity in carcinoma of the stomach. Am. J. Surg., 26: 447–456 (1934).

(*14*) HARTMAN, H. R.: The prevalence of free hydrochloric acid in cases of carcinoma of the stomach. Am. J. M. Sc., 163: 186–190 (1922).

(*15*) BRUNSCHWIG, A., SCHMITZ, R. L., and RASMUSSEN, R.: Experimental observations on achlorhydria of gastric cancer. J. Nat. Cancer Inst., 1: 481–488 (1941).

(*16*) SCHINDLER, R., NUTTER, P. B., GROOM, H., and PALMER, W. L.: Anatomic foundation of anacidity; a gastroscopic study. Arch. Int. Med., 66: 1060–1078 (1940).

(*17*) SCHINDLER, R., and MURPHY, H. M.: Symptomatology of chronic atrophic gastritis. Am. J. Digest. Dis., 7: 7–13 (1940).

(*18*) RUFFIN, J. M., and BROWN, I. W. Jr.: The occurrence of gastritis as diagnosed by gastroscopy in gastric neuroses. Am. J. Digest. Dis., 7: 414–417 (1940).

Kidney and Blood Catalase Activity of Tumor-Bearing Animals

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The liver catalase activity of rats and mice bearing a wide variety of tumors is very much lower than that of normal animals of these species (*1–5*).[1] The decrease in this activity is progressive with the growth of the tumor, the extent of the drop being most marked in animals bearing the most rapidly growing tumors.[2] Removal of the tumor results in a rapid restoration of the liver catalase activity to the normal level (*2, 4*). A few exceptions to the finding of a lowered liver catalase activity in tumor-bearing animals have been noted (*4*).

Both the blood and kidneys also have appreciable catalase activity (*7*). In order to see whether the catalase activity of these two tissues in tumor-bearing animals is affected by the presence of the tumor, determinations of this enzyme in blood and in extracts of the kidney were made.

EXPERIMENTAL PROCEDURE

The kidneys were removed from the animals immediately after decapitation, ground with sand in a mortar, and the homogenized tissue was taken up in three times its weight of distilled water. After standing for 18 hours at 5° C. in an ice box the extract was centrifuged, an aliquot of the supernatant tested for total nitrogen, and the catalase determinations were performed on appropriate dilutions of the supernatant directly after the nitrogen estimation. This technique is similar to that previously employed for enzymatic studies of the liver (*1–5*).[3] In the present study the livers were also removed and studied simultaneously with the kidneys so that a comparison of the enzymatic activity of the two tissues from the same animal was invariably obtained.

The tumor-bearing animals included dilute brown mice bearing the following transplanted tumors: S37, CR180, and malignant melanoma (*8*); dilute brown × I mice bearing the transplanted hepatoma 1 (*9*); C3H mice bearing the transplanted hepatoma (*9*), transplanted gastric adenocarcinoma (*10*), and spontaneous mammary tumors; strain A mice bearing a primary subcutaneous tumor induced by benzpyrene administration; Osborne-Mendel rats bearing the transplanted hepatoma 31 (*11*); and Buffalo rats bearing the transplanted Jensen sarcoma.[4]

It should be strongly emphasized that animals selected for studies like the present one must possess healthy and progressively growing tumors (*5*). Animals in which tumors are infected, regressing, or not fully growing, are unsatisfactory for use. Thus, the dilute brown mice bearing sarcoma 37 which we have used (*4, 5*) rarely survive beyond 2 weeks following inocula-

[1] *It is interesting to note that Brahn* (6) *found that the liver catalase activity of human beings with cancer of the stomach, intestine, rectum, pancreas, and gall bladder was very much lower than normal.*

[2] *We have observed that homologous embryonic tissue growing rapidly under the skin of C3H and dilute brown mice does not affect the liver catalase activity of the host animals.*

[3] *The 18-hour period of standing of the extracts at 5° C. is not necessary since freshly prepared extracts when tested for catalase activity yielded the same results as those which had stood for the above period.*

[4] *Dr. H. L. Stewart furnished the mice bearing gastric adenocarcinomas and Dr. M. J. Shear the mice bearing the benzpyrene-induced primary tumors.*

tion with the tumor, and at this time the tumor weighs approximately 8 gm. The effect on the liver catalase of these animals is profound (*4*, *5*). The liver catalase of dilute brown mice bearing sarcoma 37 and surviving for 3 weeks is very little depressed below normal because of the slow growth rate of the tumor, which at this time weighs about 1 gm. Similar findings on hepatoma 31 and the Jensen sarcoma in rats have been observed (*5*).

Blood catalase studies were made on normal Buffalo rats and on Buffalo rats bearing the transplanted Jensen sarcoma 2 weeks following inoculation. The animals were decapitated, and the blood was caught in a clean watch glass and used immediately for the catalase determinations. Since the catalase activity of the blood is found almost entirely in the red cells, a count of the latter, together with hemoglobin determinations, was made simultaneously on the same sample of blood used for the enzymatic determinations.

A few determinations of the catalase activity of extracts of the spleen and of the red marrow of normal and tumor-bearing rats were made. The activity of these tissues was very low and variable and appeared to be nearly wholly due to the presence of varying amounts of blood. The low catalase activity of the marrow was surprising in view of the fact that the red cells are synthesized in the marrow.

The catalase activity of the tissues described was determined by the volumetric

TABLE 1.—*Liver and kidney catalase activity of normal and tumor-bearing animals*

[Temperature 25° C., 1.0 cc. of 29 percent H_2O_2 added to mixture of 1.0 cc. extract plus 5 cc. of M/5 phosphate buffer at pH 6.9. Liver and kidney activity determined on the same animals]

Animal	Animals used	Tumor	Age of tumor[1]	Concentration of N in extract	Catalase activity, O_2 evolved in—	
					Liver	Kidneys
	Number		*Days*	*Milligram per cubic centimeter*	*Cubic centimeters per second*	*Cubic centimeters per second*
Normal dilute brown mice.	12			0.6	2.0	0.8
				.3	.8	.4
Dilute brown mice	6	Transplant S37	7	.6	.7	.5
				.3	.3	.2
Do	18	do	12	.6	.2	.4
				.3	.08	.2
Do	4	Transplant CR180	12	.6	.2	.6
				.3	.08	.2
Do	4	Transplanted melanoma	60	.6	.9	.8
				.3	.4	.4
Dilute brown × I mice.	8	Transplanted hepatoma	21	.6	.3	.7
				.3	.1	.4
Normal C3H mice	12			.6	2.0	1.0
				.3	.9	.5
C3H mice	4	Transplanted hepatoma	60	.6	1.8	.9
				.3	.9	.5
Do	4	Transplanted gastric adenocarcinoma.	21	.6	.6	1.0
				.3	.2	.4
Do	30	Spontaneous mammary	?	.6	.8	.2
				.3	.3	.1
Normal A mice	4			.6	1.8	.8
				.3	.9	.4
A mice	4	Primary induced by benzpyrene	?	.6	.6	.6
				.3	.3	.3
Normal Osborne-Mendel rats.	6			.6	1.6	1.0
				.3	.7	.5
Osborne-Mendel rats	8	Transplanted hepatoma 31	35	.6	.2	.8
				.3	.1	.4
Normal Buffalo rats	12			.6	1.8	1.3
				.3	.8	.6
Buffalo rats	24	Transplanted Jensen sarcoma	18	.6	.3	1.1
				.3	.1	.5

[1] Age of tumor refers to the time elapsed since implantation. The question marks in this column refer to the fact that the true age of the spontaneous and induced tumors is not known. The average weight of the former tumor in each mouse used in these studies was 6 gm., that of the latter, 3 gm.

method (*1–5*). The variation in activity between individual animals was about ±20 percent. The results on the kidneys and livers of normal and tumor-bearing animals are given in table 1.

The kidney catalase activity of the normal rats and mice studied is lower than that of the liver catalase of the same animals, the difference being greater in mice than in rats. Indeed, the kidney catalase activity in normal mice is about half that of the liver catalase, whereas in normal rats the activity of the two tissues is nearly of the same order (table 1).

In the tumor-bearing rats the liver catalase activity is very much lower than in normal rats (table 1 and (*2*)); on the other hand, the kidney catalase activity of the tumor-bearing rats is lowered very little. We have, therefore, the striking and unusual picture in the tumor-bearing rats of kidneys much more active in catalase than the livers of the same animals. The same condition is observed in some of the tumor-bearing mice (table 1). The kidney catalase is greater in activity than the liver catalase in mice bearing sarcoma 37, CR180, hepatoma 1, and the gastric adenocarcinoma. This is obviously due to the fact that the kidney catalase activity in these animals is depressed much less than is the liver catalase activity. The latter explanation holds also in the cases of the mice bearing the melanoma and the primary benzpyrene-induced tumor where the liver and kidney catalase activities are approximately equal. In contrast with these observations is the case of mice bearing spontaneous mammary tumors in which the kidney catalase activity is depressed considerably below that of the liver and that of the kidneys in normal mice. In these mice the liver catalase is depressed to about half the normal activity, that of the kidney to about one-fifth. Because of the exceptional findings in this instance we used a large number of animals carrying the spontaneous tumor (table 1) with uniformly consistent results. The results on the liver catalase activity in table 1 are consistent with those earlier reported (*1–5*).

TABLE 2.—*The blood catalase activity of normal rats and of rats bearing the transplanted Jensen sarcoma*

[0.1 cc. freshly obtained blood was added to 10 cc. distilled water, and 5 cc. of this solution treated with 1.0 cc. 29 percent H_2O_2 at 25° C. Red cell count, hemoglobin determinations, and catalase determinations were run simultaneously on the same sample of blood]

Animals	Red cell count	Hemoglobin	Catalase activity—evolution of oxygen
	×10³	*Grams per 100 cc. blood*	*Cubic centimeters per second*
Normal Buffalo rats:			
No. 1	6,560	11.2	1.4
2	4,769	9.9	.9
3	6,240	10.5	1.0
4	5,770	11.5	1.3
5	6,520	10.1	.5
6	5,900	11.0	.8
7	5,710	12.8	.5
8	4,140	12.8	1.4
9	7,720	14.8	.5
10	5,400	13.0	2.0
Average	5,873	11.8	
Buffalo rats bearing Jensen sarcoma 2 weeks after implantation of tumor:			
No. 1	5,820	11.5	2.0
2	3,650	10.3	1.0
3	3,680	10.8	.2
4	4,660	8.4	1.1
5	2,820	6.2	.9
6	4,290	9.0	1.4
7	3,900	10.2	1.1
8	3,220	9.5	1.0
9	4,600	10.8	1.6
10	4,080	9.5	1.2
Average	4,072	9.6	

The data on the catalase activity of the blood of normal and of tumor-bearing rats are given in table 2.

Table 2 reveals the wide variation in the blood catalase activity from one rat to the other. Such variations are not observed in determinations of the liver and kidney catalase activity where the greatest variation is ± 20 percent. The evolution of oxygen from digests of these two tissues with hydrogen peroxide is smooth and uniform. Toward the end of the relatively short digestion period the rate of evolution of gas slows down,

owing, presumably, to a gradual destruction of the enzyme. This rate curve is graphically depicted in an earlier report (*2*). In contrast, the incubation of blood with peroxide results in an abrupt evolution of oxygen which after a few seconds just as abruptly drops to an almost imperceptible rate of evolution. It would appear that the erythrocyte catalase is rapidly inactivated.[5] In spite of the individual variations, examination of the data for the two sets of animals reveals little difference in the blood catalase activity of the normal and of the tumor-bearing rats. If the results for each set are averaged, a somewhat dubious procedure in view of the wide variations, the values are about 1.0 cc. per second for the normal animals and about 1.1 cc. per second for the tumor-bearing animals.

The red-cell count and the hemoglobin concentration of the blood of the tumor-bearing rats are in general lower than those of normal rats (table 1). Nevertheless, there appears to be no correlation between these data and the catalase activity of the blood in each group.

DISCUSSION

The three catalase-active animal tissues, liver, kidney, and blood, are apparently affected to considerably different degrees in tumor-bearing animals. With few exceptions, the liver catalase activity of tumor-bearing animals which have been studied is markedly less than that of normal animals ((*1–5*) and table 1). With the exception of C3H mice bearing spontaneous mammary tumors, the kidney catalase activity of the tumor-bearing animals studied is lowered to a much less degree than is the liver catalase. This leads to the unusual situation in which the kidney catalase activity in rats and mice is considerably greater than the liver catalase activity (table 1). Finally, the blood catalase, at least in rats bearing the Jensen sarcoma, is apparently unchanged in activity despite the fact that in these animals the liver catalase activity is far below the normal level.

In earlier reports on this subject, the hypothesis was tentatively advanced that a toxic material was given off by the tumor to the general circulation and that this material accumulating in the liver resulted in a decrease in the catalase activity of this organ (*1–5*). A rapidly growing tumor such as sarcoma 37 would flood the liver with such a substance producing the effect on the catalase activity already noted (*4*). A slowly growing tumor, putting forth this toxic material slowly, would not affect the liver appreciably since presumably the latter would have sufficient time to excrete the material. To carry this hypothesis further in an attempt to explain the failure to affect markedly the kidney catalase, it is possible that the failure is due to a partial excretion of the toxic material by the kidney. In the exceptional case of the mice carrying spontaneous tumors, it is possible that the material instead of being largely excreted is resorbed into the kidney producing the eventual anticatalase effect noted in table 1. The toxic material in these mice may be somewhat different in nature than that produced by other tumors since the effect on the kidney catalase elicited by other kinds of tumors within this strain is different. The failure of the blood catalase to be affected by the presence of the Jensen sarcoma may be due to the fact that at any given instant there is very little of the toxic material to be found in the blood.

An alternative explanation, retaining the

[5] *The rate of inactivation presumably varies from one rat to the other since duplicate determinations of the catalase activity on any one blood sample agree uniformly to within 10 percent.*

hypothesis of a toxic material given off by the tumor, may be based on differences in the neutralizing power of the two tissues and the blood against this material. There is no reason to believe that all three are equally susceptible to the action of such a foreign agent despite the fact that the catalase molecule is the same in all of them. It is hardly likely that the effect of the toxic material is directly on the enzyme.

No matter what hypothesis may be advanced, however, the facts themselves are clear-cut and challenging. Since other enzymes studied in the tissues of tumor-bearing animals have not shown any difference in activity from those of normal animals (*1–5, 9, 12*), it is advisable that attention in this problem should focus on biochemical systems related to catalase. These would be chiefly the iron-containing and copper-influenced systems. The work of Schultze and Kuiken is highly suggestive in this respect (*13*).

SUMMARY

The liver and kidney catalase activities of the same animal were compared in a number of normal and tumor-bearing rats and mice. The catalase activity of the kidneys of normal mice was about half that of the livers; in normal rats the kidney catalase activity was only slightly less than that of the liver of this species. Similar to previous findings, the liver catalase activity of animals bearing rapidly growing tumors was very much less than that of normal animals. On the other hand, with the exception of C3H mice bearing spontaneous mammary tumors, the kidney catalase activity of tumor-bearing animals was lowered relatively little. In such animals, therefore, the unusual situation exists of more highly catalase active kidneys than livers. In the case of the mice bearing spontaneous mammary tumors the kidney catalase activity was lowered to a greater degree than was the liver catalase.

The blood catalase activity of rats bearing the Jensen sarcoma was indistinguishable from that of normal rats despite a lower red cell count and hemoglobin concentration in the blood of the former group of animals.

Hypotheses based on the liberation of a toxic material from the tumor into the general circulation are discussed.

REFERENCES

(*1*) GREENSTEIN, J. P., JENRETTE, W. V., and WHITE, J.: The relative activity of xanthine dehydrogenase, catalase, and amylase in normal and cancerous hepatic tissues of the rat. J. Nat. Cancer Inst., 2: 17–22 (1941).

(*2*) GREENSTEIN, J. P., JENRETTE, W. V., and WHITE, J.: The liver catalase activity of tumor-bearing rats and the effect of extirpation of the tumors. J. Nat. Cancer Inst., 2: 283–291 (1941).

(*3*) GREENSTEIN, J. P., JENRETTE, W. V., MIDER, G. B., and ANDERVONT, H. B.: The relative enzymatic activity of certain mouse tumors and normal control tissues. J. Nat. Cancer Inst., 2: 293–299 (1941).

(*4*) GREENSTEIN, J. P., and ANDERVONT, H. B.: The liver catalase activity of tumor-bearing mice and the effect of spontaneous regression and removal of certain tumors. J. Nat. Cancer Inst., 2: 345–355 (1942).

(*5*) GREENSTEIN, J. P.: Titration of the liver catalase activity of normal and of tumor-bearing rats and mice. J. Nat. Cancer Inst., 2: 525–530 (1942).

(*6*) BRAHN, B.: Weitere Untersuchungen über Fermente in der Leber von Krebskranken. Sitzungsb. d. K. Preuss. Akad. d. Wissensch., pp. 478–481 (1916).

(*7*) v. EULER, H.: Chemie der Enzyme, pt. 2., sec. 3, pp. 1–75. Munich (1934).

(*8*) GREENSTEIN, J. P., TURNER, F. C., and JENRETTE, W. V.: Chemical studies on the components of normal and neoplastic tissues. IV. The melanin-containing pseudoglobulin of the malignant melanoma of mice. J. Nat. Cancer Inst., 1: 377–386 (1940).

(*9*) GREENSTEIN, J. P., JENRETTE, W. V., MIDER, G. B., and WHITE, J.: Chemical studies on the components of normal and neoplastic tissues. V. The relative arginase activity

of certain tumors and normal control tissues. J. Nat. Cancer Inst., 1: 687–706 (1941).

(*10*) STEWART, H. L., and LORENZ, E.: Induction of adenocarcinoma of the pyloric stomach in mice by methylcholanthrene (preliminary report). J. Nat. Cancer Inst., 2: 193–196 (1941).

(*11*) EDWARDS, J. E., and WHITE, J.: Pathologic changes, with special reference to pigmentation and classification of hepatic tumors, in rats fed *p*-dimethylaminoazobenzene (butter yellow). J. Nat. Cancer Inst., 2: 157–184 (1941).

(*12*) GREENSTEIN, J. P.: Distribution of acid and alkaline phosphatase in tumors, normal tissues, and the tissues of tumor-bearing rats and mice. J. Nat. Cancer Inst., 2: 511–524 (1942).

(*13*) SCHULTZE, M. O., and KUIKEN, K. A.: The effect of deficiencies in copper and iron on the catalase activity of rat tissues. J. Biol. Chem., 137: 727–734 (1941).

Riboflavin Content of Tumor Tissues

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The concentration of riboflavin in liver tumors, produced by feeding 2-amino-5-azotoluene (*1*) or dimethylaminoazobenzene (butter yellow) (*2*, *3*), has been found to be significantly lower than in normal liver. In view of the occurrence of riboflavin as the functional group of several important metabolic enzyme systems and because of its striking growth-promoting effect, it was considered desirable to compare the riboflavin content of primary and transplanted hepatomas with rapidly growing nonneoplastic tissue of similar origin, i. e., regenerating liver. The results of these comparative studies, the riboflavin concentration in several other types of tumors, and the effect of a transplanted tumor on the apparently normal liver of the host, are reported in this paper.

METHODS

The rats and mice used were fed a standard Purina dog-chow diet, which they were allowed to eat ad libitum unless otherwise noted. Various methods were employed to kill the animals, asphyxiation, decapitation, or cervical dislocation. The results obtained were not affected by these differences in procedure. The tumors and tissues were removed immediately after death, freed as far as possible from necrotic tissue, rapidly blotted with filter paper to remove superficial blood or exudate, and placed in covered weighing bottles. Total solids in the tissues were determined by heating in air to constant weight at 100°–110°.

A fluorometric method was employed in the estimation of riboflavin. Light from an H–3 mercury arc was passed through a condensing lens and Corning filter combination No. 038 and No. 511 which cuts out the λ 3660A ultraviolet and all light of wavelength longer than λ 4600A (checked by a Hilger spectrophotometer). The blue λ 4360A line is superior to the ultraviolet λ 3660A as exciting radiation since it keeps the fluorescence of "interfering pigments" to a minimum. The riboflavin fluorescence excited by the blue line was passed through an Eastman flavazine No. 16 filter which cuts off sharply at λ 5200A, thus assuring that any scattered blue light did not fall on the photo cell. The maximum response of the Weston photronic cell used is fortunately not far from the λ 5600A maximum of the flavin fluorescence, thus giving a selective response for this emission. The photoelectric current was measured with a sensitive galvanometer. A sliding rack permitted rapid successive measurement of the fluorescence intensity of three samples which were contained in bottles silvered on the bottom and three sides to increase the intensity of emitted light. These had been adjusted to produce equal fluorescence when containing a standard riboflavin solution. The estimation of fluorescence intensity due to riboflavin was based on the difference in fluorescence before and after quenching with NaOH or HCl. Although either high acidity or high alkalinity completely destroys the riboflavin fluorescence (*4*), the former decreases whereas the latter increases fluorescence ascribable to other constituents in the tissue extracts. In these studies the magnitude of the interfering fluorescence was not great and it was considered safe practice to average the two differences.

The riboflavin extracts were prepared

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by autoclaving 200 to 2,000 mg. of tissue with about 25 ml. of N/10 HCl 15 minutes at 20 pounds pressure. Before autoclaving it was only necessary to mince these tissues with a glass rod since it was found that extracts of comparable samples which had been well ground with sand yielded identical results. The acid digests were brought to about pH 5–6 with N/1 NaOH, then centrifuged. An aliquot containing 1 to 10 micrograms of riboflavin was diluted to 100 ml. This fivefold to twentyfold dilution yielded solutions with practically no interfering absorption. Thirty-five milliliters was placed in each of two silvered bottles and a standard riboflavin solution in the middle bottle. Four alternate readings of fluorescence intensity were made in rapid succession. The riboflavin fluorescence was then quenched in one unknown by addition of 1 ml. 10N HCl and in the other by addition of 1 ml. 10N NaOH. Either HCl or NaOH was used to extinguish the fluorescence of the riboflavin standard. A series of four alternate readings was now made on these solutions. The changes in fluorescence intensity of the unknowns caused by HCl and NaOH were averaged and compared with that observed in the standard. The results were reproducible within ± 5 percent. Recoveries were satisfactory, and the values obtained for normal tissues agreed with those obtained by other methods.

RESULTS

It is known that riboflavin is found both in the free state and as the phosphoric ester combined with protein. Free riboflavin was determined by extracting the tissues with water at room temperature and was found to be about 25 percent of the total in both hepatoma and liver.

The data presented in table 1 show that the concentration of riboflavin in primary or transplanted liver tumors of the Osborne-Mendel rat is considerably below that of normal liver. Since tumor tissue contains more water than does normal liver, the riboflavin content has been expressed on both a dry- and a fresh-weight basis. On either basis the normal liver concentration is three to six times that of the tumor. This is obviously not due solely to the rapidity of growth because regenerating liver, which proliferates at a much greater rate than the tumor, has the same riboflavin concentration as normal liver. The similarity of tumor and fetal tissue has been often mentioned, and it is interesting that the riboflavin concentrations of tumor and fetal liver lie in the same range. This phenomenon may, however, not depend on a similarity between fetal liver cells and tumor cells but possibly on the fact that the fetal liver contains an abundance of blood-forming elements which are absent from normal adult liver.

This range of concentrations, from 20 to 30 micrograms of riboflavin per gram of dry tumor, is shared by several other neoplasms (table 2). No correlation between

TABLE 1.—*Riboflavin concentration in liver and liver tumor*

Tissue	Number of tissues	Total solids	Riboflavin	
			Fresh tissue	Dry tissue
		Mean and standard deviation	Mean and standard deviation	Mean and standard deviation
		Percent	*Micrograms per gram*	*Micrograms per gram*
Normal liver	8	30.0±0.9	29.4±2.3	98.0±5.6
Hepatic carcinoma induced by dimethylaminoazobenzene (*5*) [1]	6	19.9±2.3	4.0±1.5	20.5±3.8
Hepatoma 31 (*6*) [1]	18	17.9± .5	4.8± .4	26.7±2.6
Fetal liver	2	22.5 —	5.5±1.1	24.4 —
Regenerating liver (24–72 hours)	6	24.4±2.8	24.7±1.8	101.4±9.2
Liver of rat bearing hepatoma 31	9	26.5± .8	21.1±2.4	79.4±7.2

[1] Italic number in parentheses refers to literature reference in which the tumor is described.

either rate of growth, cell of origin, or gross appearance and riboflavin concentration of the tumors is evident. Rather the riboflavin concentration seems to be characteristic for most tumors. The mouse hepatomas, whether spontaneous or induced by 2-amino-5-azotoluene or carbon tetrachloride, are the only tumors studied containing significantly higher amounts of riboflavin. The high degree of differentiation and a marked histologic resemblance to the parent cell are pathologic features which tend to set off this tumor (*12*). The riboflavin concentration of 2-amino-5-azotoluene-induced hepatomas in the rat is also high (*1*), and these tumors present a pathologic picture similar to the mouse hepatoma but unlike the usual rat hepatoma induced by feeding dimethylaminoazobenzene.

In considering the percentage of total solids in tumor tissue (table 2), mouse hepatomas were found to average 25 percent, which is more nearly that of normal tissue than is the 17 to 20 percent total solids of other neoplasms. Although total solids of 17 to 20 percent agrees with similar values found by Cramer (*13*), a correlation between total solids and rate of growth such as he reported was not observed in these experiments.

It must be emphasized that the concentrations of riboflavin reported in tables 1 and 2 were determined on animals eating the same diet. Removal of riboflavin from the diet of normal animals has been shown to reduce the concentration in the tissues by as much as 60 percent (*15*). Table 3 shows the effect of a riboflavin-deficient diet on the concentration of this substance in two different tumors and in liver and muscle of the tumor-bearing animals. From these data it is clear that the riboflavin concentration in tumors reflects the diet to about the same degree as does that in normal tissues.[1]

[1] *A detailed report of this work including the effect on rate of growth of the tumors is reported elsewhere* (16).

TABLE 2.—*Riboflavin concentration in various tumor tissues of the rat and mouse*

Animal	Tumor	Number of tumors	Total solids	Riboflavin	
				Fresh tumor	Dry tumor
			Mean and standard deviation	Mean and standard deviation	Mean and standard deviation
			Percent	*Micrograms per gram*	*Micrograms per gram*
Rat:					
Osborne-Mendel	Hepatoma 31	18	17.9±0.5	4.8±0.4	26.7± 2.6
Buffalo	Jensen sarcoma	6	17.2± .1	3.6± .5	21.0± 2.4
Mouse:					
A	Lymphosarcoma[1]	4	18.0± .7	3.7± .1	20.6± 1.3
	Intestinal carcinoma (7)	3	19.5± .3	3.9± .2	20.2± .8
	Salivary gland tumor[2]	4	18.2±1.7	3.5± .3	19.2± 3.3
	Fibrosarcoma of the ear induced by UV irradiation (8)	5	19.9±1.8	4.4± .4	22.1± 1.3
dba	Malignant melanoma S91	14	20.2±1.5	5.6± .9	27.8± 5.0
	Sarcoma 37	4	18.6± .9	3.7± .4	20.2± 3.1
	Crocker sarcoma 180	4	17.7± .8	4.1± .8	23.2± 5.4
C3H	Brain tumor (9)	6	9.5±.9	2.4±1.2	17.4± 2.9
	Spontaneous mammary adenocarcinoma	5	18.8±1.7	5.5± .6	29.5± 3.6
	Adenocarcinoma of the glandular stomach (10)	6	18.4±1.8	4.3± .3	23.5± 1.8
C57 Brown	Squamous-cell carcinoma of the forestomach (11)	6	17.8± .8	3.3± .3	18.7± 1.6
C3H, I, and C57 Black	Hepatoma induced by azotoluene[3]	29	23.6±1.5	8.4±3.0	47.1±13.0
C3H, A	Spontaneous hepatoma (12)	10	25.7±1.4	15.3±1.4	51.4±12.7
A	Hepatoma induced by carbon tetrachloride (13)	7	22.3±1.5	15.2±4.1	56.7±16.4

[1] 18th generation transplants of a spontaneous neoplasm arising in a lymph node.
[2] A spindle-cell tumor arising in the region of the salivary glands in the neck, possibly of basket-cell origin.
[3] Rapidly growing, well-differentiated tumor of hepatic-parenchymal-cell origin.

TABLE 3.—*Effect of riboflavin concentration in the diet on the concentration in tumors*

Animal	Riboflavin per gram dry tissue in—			
	Spontaneous adenocarcinoma	Transplanted hepatoma 31	Liver	Muscle
	Micrograms	*Micrograms*	*Micrograms*	*Micrograms*
C3H mouse:				
Diet high in riboflavin	29.5	.....	120.7	16.3
Diet deficient in riboflavin	16.5	.....	64.5	11.1
Osborne-Mendel rat:				
Diet high in riboflavin	.....	29.9	108.8	17.8
Diet deficient in riboflavin	.....	21.4	56.9	8.9

Table 1 shows that the riboflavin concentration and the percentage total solids are significantly lower not only in hepatoma 31 but also in the apparently normal livers of animals carrying this neoplasm than they are in the livers of normal rats. The livers of animals bearing tumors were, therefore, analyzed. As may be seen from table 4 the percentage total solids is distinctly lower in the livers of each of the tumor-bearing groups than in normal liver which confirms similar observations made by Medigreceanu (*17*).

TABLE 4.—*Total solids in the livers of tumor-bearing rats and mice*

Tumor	Number of animals	Total solids
		Mean and standard deviation
		Percent
Rat:		
Controls	11	29.9±1.0
Jensen sarcoma	6	25.6±1.8
Hepatoma 31	9	26.5± .8
Mouse:		
A:		
Controls	9	30.9±2.1
Lymphosarcoma	8	22.8±1.5
Intestinal carcinoma	3	26.1±1.3
Salivary gland tumor	2	26.2±2.0
Fibrosarcoma of the ear induced by UV irradiation	5	28.4±1.2
dba:		
Controls	4	30.8± .6
Malignant melanoma 891	9	27.1±1.3
Sarcoma 37	4	27.4±1.5
Crocker sarcoma 180	4	27.9± .2
C3H:		
Controls	4	31.2± .3
Brain tumor	6	27.9±1.6
Spontaneous mammary adenocarcinoma	5	27.6±1.1
Adenocarcinoma of the glandular stomach	6	27.1±1.2
Hepatoma	3	27.0±1.9
Malignant hemangioendothelioma	2	27.6± .7
C57 Brown: Squamous-cell carcinoma of the forestomach	6	27.2±1.1

The riboflavin concentration in the livers of animals bearing tumors other than hepatoma 31 is, however, in no case significantly lower than in normal liver. Indeed, the riboflavin concentration was found to be higher in the liver of strain A mice bearing a fibrosarcoma of the ear induced by ultraviolet irradiation than in normal liver. Such variations seem to preclude the possibility that tumors in general exert a systemic effect on liver riboflavin of the same order of magnitude as that observed for liver catalase (*18*).

DISCUSSION

The availability of a hepatoma of known liver-cell origin has made possible this study of a biochemical change which a specific tissue undergoes upon becoming cancerous. The fundamental difference in the metabolism of normal and neoplastic tissues first observed by Warburg must be due to quantitative or qualitative changes in enzyme distribution. Since riboflavin is the prosthetic group of several enzymes involved in metabolic processes, any significant change in its concentration must affect the mode of energy turnover of the tissue. It is well, therefore, to have the difference in riboflavin concentration between normal liver and liver tumor established beyond doubt. As has been previously pointed out, calculation of the riboflavin concentrations on either a dry- or fresh-weight basis does not alter the finding that, gram for gram, liver contains three to six times as much riboflavin as does hepatoma.

Since liver is often thought of as the "body's storehouse" in which case only part of the total riboflavin would be functionally active, the possibility that the

observed differences in hepatoma and liver are due to stored riboflavin must be considered. However, even the lowest riboflavin concentration found in the depleted liver of rats on a deficient diet is still twice that in hepatomas in animals ingesting a high riboflavin diet. These findings demonstrate conclusively that the low riboflavin concentration of hepatoma is not an artifact but a real indication of a different type metabolism. The most pertinent question arising from this observation is whether all flavin-containing enzymes are proportionately lower in tumors or whether some are unaffected and others completely lacking. A partial answer is supplied by the observation of Greenstein who found that the flavin enzyme, xanthine dehydrogenase, present in tumors is about 50 percent of that found in normal liver (*19*).

In view of the well-known effect of riboflavin as a growth stimulant, it is surprising that the riboflavin concentration of different tumors was not related to rate of growth, but seemed to be a characteristic value for most tumors. Since the mouse hepatomas and the *o*-aminoazotoluene-induced rat hepatoma (*1*) were the only tumors analyzed containing significantly greater amounts of riboflavin and since these tumors present a unique pathologic picture, the two observations may be related. These hepatomas have not been proven malignant, whereas all of the other tumors analyzed in this series have been. It is possible that the riboflavin concentration may depend on whether the neoplasm is benign or malignant or upon the degree of malignancy.

SUMMARY

A fluorometric method for the determination of riboflavin in tissues is described.

The riboflavin concentration in hepatoma and in fetal, normal, and regenerating liver were found to be, respectively, 25, 25, 100, and 100 micrograms per gram of dry tissue.

A concentration of 20–30 micrograms of riboflavin per gram of dry tissue was, with one exception, characteristic of the tumor tissues studied. Of 15 different tumors analyzed, only the mouse hepatoma fell outside this range.

The total solids of 12 tumors ranged from 17 to 20 percent. In the mouse hepatoma total solids averaged 24 percent, and in the glioma 10 percent.

The grossly normal livers of all tumor-bearing rats and mice had less total solids than the livers of normal controls. The riboflavin, however, was found to be lowered only in the rats bearing hepatoma 31.

REFERENCES

(*1*) KAHLER, H., and DAVIS, E. F.: Riboflavin determinations on normal liver and liver tumor. Proc. Soc. Exper. Biol. & Med., 44: 604–606 (1940).

(*2*) KENSLER, C. J., SUGIURA, K., and RHOADS, C. P.: Coenzyme I and riboflavin of livers of rats fed butter yellow. Science, 91: 623 (1940).

(*3*) MASAYAMA, T., and YOKOYAMA, T.: Biochemisches Studium über das Krebsgewebe der Leber. Gann, 33: 214–216 (1939).

(*4*) KUHN, R., and MORUZZI, G.: Die Dissoziation des Lactoflavins . . . Ber. d. deutsch. chem. Gesellsch., 67B: 888–891 (1934).

(*5*) EDWARDS, J. E., and WHITE, J.: Pathologic changes, with special reference to pigmentation and classification of hepatic tumors in rats fed *p*-dimethylaminoazobenzene (butter yellow). J. Nat. Cancer Inst., 2: 157–183 (1941).

(*6*) WHITE, J., DALTON, A. J., and EDWARDS, J. E.: Pathology of rat hepatoma 31. J. Nat. Cancer Inst., 2: 539–554 (1942).

(*7*) LORENZ, E., and STEWART, H. L.: Intestinal carcinoma and other lesions in mice following oral administration of 1,2,5,6-dibenzanthracene and 20-methylcholanthrene. J. Nat. Cancer Inst., 1: 17–40 (1940).

(*8*) GRADY, H. G., BLUM, H. F., and KIRBY-SMITH, J. S.: Pathology of tumors of the external ear in mice induced by ultraviolet radiation. J. Nat. Cancer Inst., 2: 269–276 (1941).

(9) SELIGMAN, A. M., and SHEAR, M. J.: Studies in carcinogenesis. VIII. Experimental production of brain tumors in mice with methylcholanthrene. Am. J. Cancer, 37: 354–395 (1939).

(10) STEWART, H. L., and LORENZ, E.: Induction of adenocarcinoma of the pyloric stomach in mice by 20-methylcholanthrene (preliminary report). J. Nat. Cancer Inst., 2: 193–196 (1941).

(11) LORENZ, E., and STEWART, H. L.: Squamous-cell carcinoma and other lesions of the forestomach in mice following oral administration of 20-methylcholanthrene and 1,2,5,6-dibenzanthracene (preliminary report). J. Nat. Cancer Inst., 1: 273–276 (1940).

(12) EDWARDS, J. E., DALTON, A. J., and ANDERVONT, H. B.: Pathology of a transplantable spontaneous hepatoma in a C3H mouse. J. Nat. Cancer Inst., 2: 555–563 (1942).

(13) EDWARDS, J. E.: Hepatomas in mice induced with carbon tetrachloride. J. Nat. Cancer Inst., 2: 197–199 (1941).

(14) Cramer, W.: On the biochemical mechanism of growth. J. Physiol., 50: 322–334 (1916).

(15) FRAZER, H. F., TOPPING, N. H., and ISBELL, H.: The bacterial assay of riboflavin in the urine and tissues of normal and depleted dogs and rats. Pub. Health Rep., 55: 280–289 (1940).

(16) MORRIS, H. P., LIPPINCOTT, S. W., and ROBERTSON, W. V. B.: Increase in the number and rate of growth of spontaneous mammary carcinomas caused by riboflavin in C3H mice. Paper presented in the Proceedings of the American Association for Cancer Research, Boston, April 1942.

(17) MEDIGRECEANU, F.: Über die Grössenverhältnisse einiger der wichtigsten Organe bei tumortragenden Mäusen und Ratten. Berlin klin. Wchnschr., 47: 588–593 (1910).

(18) GREENSTEIN, J. P., and ANDERVONT, H. B.: The liver catalase activity of tumor-bearing mice and the effect of spontaneous regression and of removal of certain tumors. J. Nat. Inst., 2: 345–355 (1942).

(19) GREENSTEIN, J. P., JENRETTE, W. V., and White, J.: The relative activity of xanthine dehydrogenase, catalase, and amylase in normal and cancerous hepatic tissues of the rat. J. Nat. Cancer Inst., 2: 17–41 (1942).

Pathologic Changes Associated with Riboflavin Deficiency in the Mouse

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The synthesis of crystalline riboflavin has made it possible to investigate, under carefully controlled conditions, the pathologic changes associated with acute ariboflavinosis and chronic partial ariboflavinosis. Since the mouse is so widely used in cancer research, it was chosen for these experiments which form part of our general plan of studying the nutritional requirements and pathologic changes associated with nutritional deficiencies in this species.

Young mice fed a diet lacking in riboflavin failed to grow normally and died within 6 to 8 weeks. Adult mice maintained on a similar regimen lost weight gradually and succumbed within 8 and 10 weeks. These two groups died in what is considered as acute ariboflavinosis. If either young or adult mice in acute ariboflavinosis were given minimal sustaining amounts of riboflavin in their diets, they lived up to 10 months in a state of chronic partial deficiency. Lesions involving the skin and eye developed in both groups, and lesions in the nervous system occasionally in the acute-deficiency group and frequently in the chronic-deficiency group. The morphologic character of these lesions is described in this report and is compared with lesions in other species thus far studied by different investigators.

EXPERIMENTAL PROCEDURE

Young male and female mice at the time of weaning and adult male and female mice 3 months old were employed in these experiments. C3H mice were used exclusively. A total of 135 mice were used, of which 20 comprised the control group. The control group of young and adult mice received diet 67, which contained 10.0 mg. of crystalline riboflavin added to each kilogram of the following basal diet:

	Grams
Casein (Labco, vitamin-free)	18.0
Dextrose	51.3
Crisco	10.0
Salt mixture (185a, McCollum's No. 185 modified as to calcium and trace elements)	4.5
Choline hydrochloride	.2
Wheat-germ oil	.5
Cod-liver oil	3.0
Corn oil (Mazola)	10.0
Ryzamin B (a rice polish concentrate containing not more than a trace of riboflavin)	2.5
Total	100.0

The young and adult mice in the acute-deficiency experiments received diet 66 which was identical with diet 67, save that no crystalline riboflavin was added to it. Diet 66 was also fed to another group of young mice until an acute deficiency state was produced, and then sufficient riboflavin was administered to permit the animals to live in a state of chronic partial ariboflavinosis.

A complete autopsy was performed on each animal. The tissues were fixed in dilute formalin (U. S. P. 1:10), Zenker's fluid, absolute ethyl alcohol, and Müller's fluid. The stains were haematoxylin and eosin, van Gieson's stain for connective tissue, Foot's modification of Bielschowsky's method for reticulum, Verhoeff's stain for elastic tissue, Mallory's phosphotungstic

acid haematoxylin stain, Best's carmine stain for glycogen, potassium ferrocyanide-hydrochloric acid stain for iron, sudan IV stain for fat, Sato's peroxidase reaction, Giemsa's stain, eosin methylene stain, Weigert-Pal stain, and Marchi's method for fatty degeneration of the nervous system.

The following were examined microscopically: eye, salivary gland, thyroid gland, heart, lung, stomach, small and large intestine, liver, kidney, adrenal gland, pancreas, bladder, testis, prostate, seminal vesicle, ovary, uterus, lymph node, spleen, femur, vertebra, sternum, skin, brain, spinal cord, sciatic nerve, and skeletal muscle.

CLINICAL OBSERVATIONS AND GROSS PATHOLOGY

Young mice placed on control diet 67 grew nearly as well as mice of similar age placed on a satisfactory stock diet composed of natural foodstuffs. No clinical evidence of any lesion was noted in this control group. In contrast the growth of young mice on deficient diet 66 was greatly retarded, as judged by size and body weight. The hair of these animals became thinned about the snout and ears, over the region of the scapulae, and occasionally on the forelegs. Superficial cracks and, later, deep fissures were seen about the snout and on the inner surface of the front legs. Terminally, a few animals developed a sticky exudate on the conjunctivas which partially closed the eyelids. The maximum length of life for this group was 8 weeks. The adult mice placed on diet 66 gradually lost about one-third of their normal weight, and none survived over 14 weeks. The appearance of the skin and eyes of these mice was similar to that of the young mice on the same regimen.

After 3 or more months, the mice in a state of chronic partial ariboflavinosis appeared smaller than normal when compared with the control animals. The cutaneous lesions observed in the adults in acute ariboflavinosis also developed in these animals but became more intensified. The skin in the hairless areas became dry and tough. Fissures formed about the mouth and became encrusted by serous exudate. The ears became thickened and distorted (fig. 1, *A*). When standing the animals assumed a hunched position and sometimes on walking exhibited a slightly dragging, jerky gait (fig. 1, *B*) with mild ataxia. Keratosis and cataracts were observed but were uncommon.

Examination of the internal viscera at autopsy disclosed no obvious lesions in any of the groups, but in general the organs appeared smaller than normal.

HISTOPATHOLOGY

Lesions were observed only in the skin, nervous system, and eyes.

Skin

The earliest cutaneous lesion was a simple focal atrophy of the epidermis, sometimes leaving the corium entirely exposed as a result of desquamation. On either side of such an area of desquamation the epithelium appeared normal. Occasionally areas devoid of epithelial cells showed thick layers of keratin resting directly upon the collagen fibers of the corium. More often hyperkeratosis developed without any notable preceding epithelial atrophy. In such instances, the keratin contained within it great numbers of degenerating cells, often forming amorphous detritus (fig. 2, *A*). The corium remained intact. The sebaceous glands did not atrophy and continued to be filled with a fatty substance.

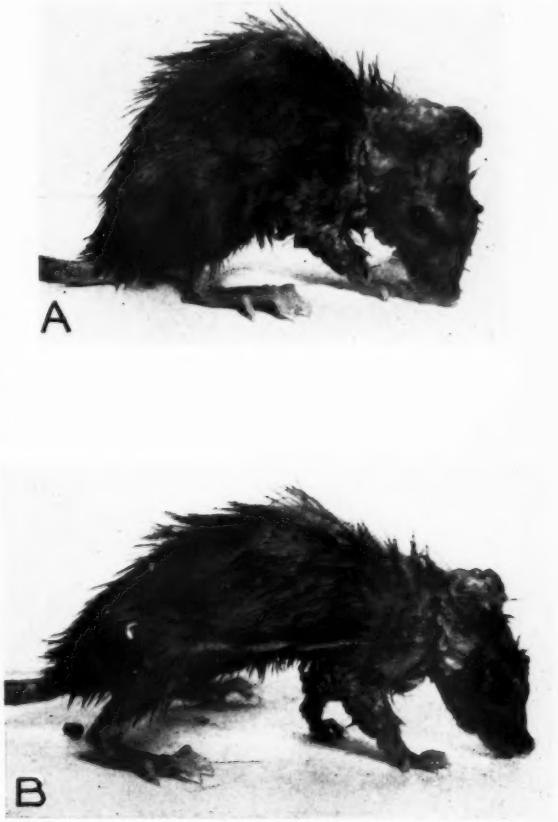

FIGURE 1.—Clinical appearance of an adult mouse in chronic partial ariboflavinosis. *A*, Cutaneous lesions with marked distortion of ears. At rest the animal remains in this hunched position; *B*, Dragging of the hind legs by the same animal when walking.

Intraepithelial aggregations of cells were observed (fig. 2, *B*), but it was impossible to determine their origin. In the peripheral portion of such accumulations, some of the cells appeared to be detached from adjacent viable squamous epithelial cells. Centrally, nuclei of many cells appeared lobated as though of polymorphonuclear leukocytes. However, cytoplasmic granules were not demonstrated by Sato's method or by the Giemsa technique. Furthermore, leukocytes were not found in the underlying corium. No identifiable lymphocytes, plasma cells, histiocytes, or red blood cells were associated with these intraepithelial cellular collections.

Large atrophic areas and often ulceration developed about the snout during the state of chronic partial ariboflavinosis (fig. 3, *A*). Most of the ulcers were shallow, and their margins were not elevated. The bases extended irregularly into the corium and subcutaneous tissue with disruption of collagen fibers. Inflammatory exudate was small in amount when present and then only in ulcers of relatively long standing (fig. 3, *B*). In no instance was an ulcer initiated by an inflammatory process.

NERVOUS SYSTEM

Sagittal, coronal, and horizontal sections of cerebrum and cerebellum from deficient

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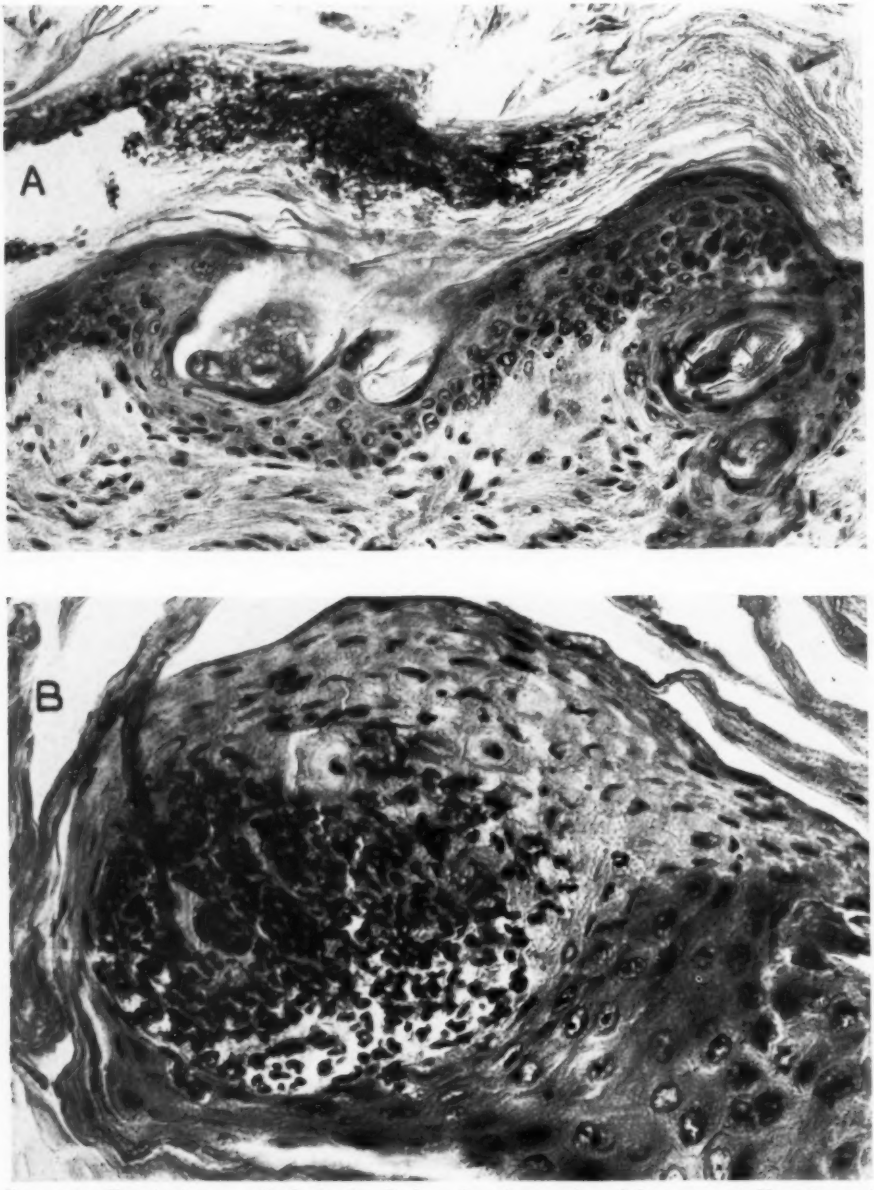

FIGURE 2.—Terminal acute ariboflavinosis. Both sections from region of the snout. *A*, Hyperkeratosis engulfing nuclear detritus. × 375; *B*, Intraepithelial cellular accumulation. × 450.

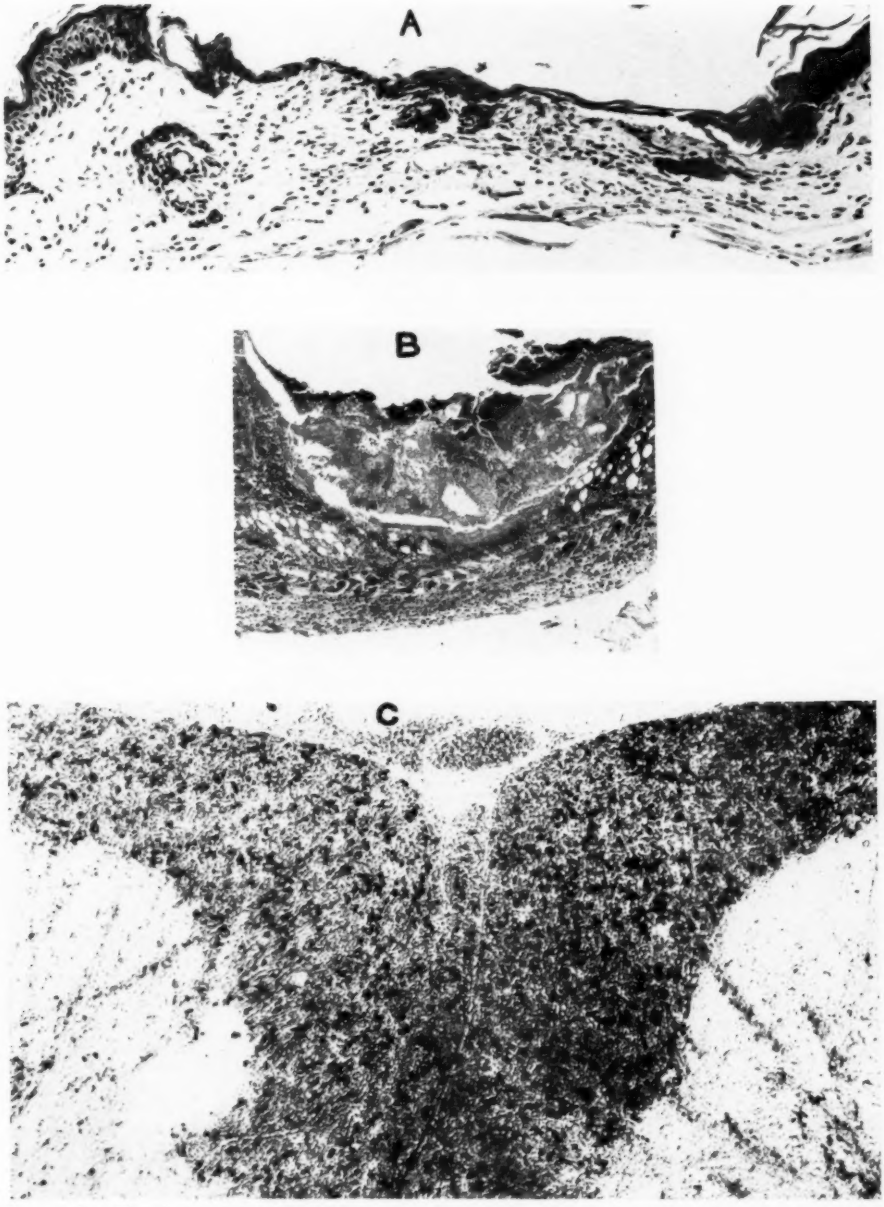

FIGURE 3.—Chronic partial ariboflavinosis. *A*, Atrophy of epidermis in region of snout. × 160; *B*, Cutaneous ulcer with inflammatory exudate in base. × 80; *C*, Myelin degeneration in the posterior columns of the spinal cord (Marchi technique). × 160.

animals appeared normal with the haematoxylin and eosin stain. Occasionally, with the Marchi technique, a few irregular black specks were found in the cerebellum but were not confined to the nerve fiber sheaths. This was not a constant finding and was considered an artifact due possibly to slow penetration of the fixative. This feature was also seen in some of the control animals. Marchi preparations of the cerebrum were normal.

Only a very few mice in late acute ariboflavinosis showed pathologic changes. The spinal cords were sectioned at various levels, and with the Marchi technique black droplets were seen scattered throughout the white matter. The destruction was diffuse and not concentrated in any specific columns. The findings are compatible with what is regarded as definite evidence of myelin degeneration. With corresponding sections prepared by the Weigert-Pal method, a very minimal loss of myelin in the white matter was noted occasionally.

In the sciatic nerves, with the Marchi technique, discrete black droplets were also found in a number of chronic-deficient animals.

Eye

In a number of mice the conjunctiva was normal, but there was an irregular loss of epithelial cells of the cornea resulting in small defects. Amorphous debris overlaid such regions, and the underlying portion of Bowman's membrane appeared thickened. The substantia propria was widened and contained flattened histiocytes squeezed into cleftlike spaces. Capillary vascularization was present (fig. 4, *B*). The capillaries contained many red blood cells and leukocytes. Frequently the endothelial lining was no longer present. The lesion in the cornea was a keratitis. The anterior and posterior chambers were free from inflammatory cells. The sclera, iris, ciliary body, choroid, and retina were intact.

In some animals cataracts were found (fig. 4, *A*). The continuity of the capsule of the lens was broken, and both degeneration and minimal proliferation of the epithelial cells occurred. There was disappearance of many lens fibers in local areas forming a homogeneous, structureless material. This destroyed the usual pattern of lenticular fibrillation. Some of the disintegrating fibers formed Morgagni's globules. Phagocytes were infrequently observed. Giant cells and calcification were not found in these sections. The opacity in these eyes was due to the presence of the capsulolenticular type of cataract.

DISCUSSION

From the present literature it is difficult to appreciate the distinctive characteristics of the lesions attributed to deficiency of the individual components of the vitamin B complex. In this discussion are reviewed the lesions already described by others for deficiencies in riboflavin, pantothenic acid, and pyridoxine in the rat, and they are compared with what we have observed in the mouse. Mention of lesions arising in other species from these particular deficiencies is also made. From these data it can be ascertained whether there is any specificity in the type of lesions resulting from deficiency of each of these vitamins in the rat and mouse and other species.

A cutaneous lesion arising in young and adult rats fed a synthetic diet deficient in riboflavin was described by Sullivan and Nicholls (*1*). After 6 weeks the hair appeared uneven and ragged, and occasionally there was mild scaling on the trunk. Areas of alopecia developed later. Microscopically, the lesion was characterized by atrophy of the skin and appendages without accompanying or preceding in-

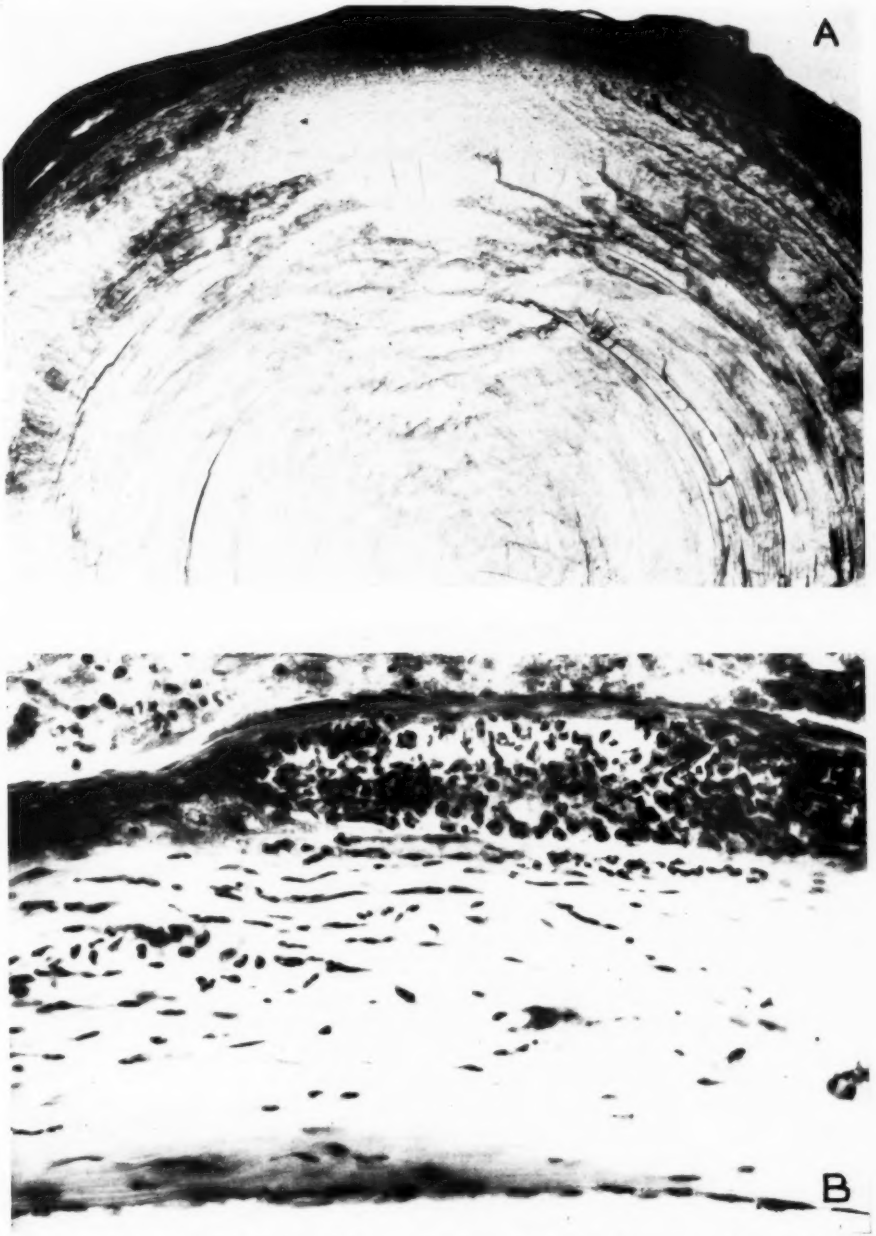

FIGURE 4.—Chronic partial ariboflavinosis. *A*, Capsulolenticular type of cataract. × 80; *B*, Keratitis with vascularization of cornea and exudative and proliferative reaction. × 400.

flammation. The lesion was essentially degenerative in character rather than inflammatory and, therefore, was similar in genesis to what was observed in our mice in the acute deficiency state. The effects of chronic deficiency upon the skin of the rat have not been reported.

Rats maintained on a diet deficient in pantothenic acid developed rusty spots on the head and back with thinning of the hair and roughening of the skin throughout, according to Unna (*2*). Sores developed about the mouth, and the whiskers became blood-caked. With scaling of the skin, baldness appeared. No histopathologic study was made of the skin of these animals. In unpublished experiments upon rats, we rarely observed thinning of the hair over the scapulae and no lesions about the mouth. Microscopically, sections from these areas of thinning occasionally showed minimal atrophy of epithelium. However, in mice deficient in pantothenic acid (*3*), we noted thinning of the hair, baldness, and scaling of the epidermis about the snout, and over the scapulae and flanks. The cutaneous lesion appeared microscopically to be an atrophic, desquamative, and at times hyperkeratotic dermatosis.

Pyridoxine deficiency in rats was reported by Sullivan and Nicholls (*4*) as resulting in symmetric dermatitis of the paws. The microscopic changes consisted of hyperkeratosis, acanthosis, edema of the corium with hyperemia and exudation, and alopecia of inflammatory origin. In mice (in as yet unpublished experiments) we never observed any cutaneous lesions in acute pyridoxine deficiency.

This comparison of results upon the skin shows that in acute riboflavin deficiency the rat and the mouse both develop a similar cutaneous lesion which is essentially degenerative rather than inflammatory in origin and that in the mouse in the chronic deficiency state the lesion is more severe. In pantothenic acid deficiency in the rat the atrophy of the skin is minimal, whereas in the mouse it is marked. Thus the dermatologic changes in riboflavin and pantothenic acid deficiencies are of similar pathologic nature in both the mouse and the rat. Pyridoxine deficiency results in a symmetric dermatitis in the rat which has no counterpart in the mouse (*5*). It appears, then, that the cutaneous lesion in riboflavin and pantothenic acid deficiencies is a dermatosis, but in pyridoxine deficiency it is a dermatitis.

The effect upon the nervous system of rats on a riboflavin-low diet was described by Engel and Phillips (*6*). Routine examination of the spinal cord and peripheral nerves showed them to be normal. Subsequently, Shaw and Phillips (*7*) observed myelin degeneration in the sciatic nerve of rats maintained on a riboflavin-deficient diet for a longer period than the animals of Engel and Phillips. Prior to these two investigations, Bethke, Record, and Kennard (*8*) showed that riboflavin preparations prevented a certain type of nutritional paralysis in chicks. Phillips and Engel (*9*) later examined the clinical and pathologic changes in chicks deficient in riboflavin. Their chicks developed ataxia, paralysis, and a peculiar inward curvature of the toes. Myelin degeneration and axis-cylinder swelling were present in the spinal cord and sciatic and brachial nerves.

Sebrell and Onstott (*10*) placed dogs upon a riboflavin-deficient diet and reported nerve-cell degeneration in the brain and spinal cord as well as myelin degeneration in certain levels of the pyramidal tracts of both brain and spinal cord. Street, Cowgill, and Zimmerman (*11*) noted the presence of myelin degeneration in the spinal cord and sciatic nerves of dogs maintained on a diet deficient in riboflavin. Patek,

Post, and Victor (*12*) patterned a diet closely similar to that used by Sebrell and Onstott and fed it to pigs. No significant changes were found on microscopic examination of the brain, spinal cord, or sciatic nerves, but clinically these animals developed spastic paralysis which was attributed by these investigators to lack of other food factors.

From the literature it has been shown that myelin degeneration has been observed in the spinal cord and peripheral nerves of the rat, chick, and dog maintained in a state of riboflavin deficiency. Myelin degeneration also occurred in our mice but was minimal, particularly when compared with that resulting in mice of the same strain placed upon a synthetic diet lacking only in pantothenic acid. In the mouse, pantothenic acid appears to be more important than riboflavin in the preservation of the nervous system. The results of pantothenic acid deficiency upon other species have not been reported. Pyridoxine deficiency has not been cited as having any definite effect upon the nervous system.

Pathologic changes occurring in the eye as a result of riboflavin deficiency were first pointed out by Langston, Day, and Cosgrove (*13*) in young albino mice. Their animals developed keratitis in an average of about 6 weeks and cataracts about a week later. Later, Bessey and Wolbach (*14*) observed a similar keratitis in rats and infrequently cataracts. Patek, Post, and Victor (*12*) detected vascularization in the cornea as well as cataracts in pigs maintained on a riboflavin-deficient diet. In man, in a small group of patients known to be receiving insufficient riboflavin, ocular lesions were observed by Kruse, Sydenstricker, Sebrell, and Cleckley (*15*). Slit-lamp examination and ocular signs showed the principal manifestation to be a keratitis. In our mice keratitis developed in the acute-deficiency, and occasionally cataracts in the chronic-deficiency state. Pantothenic acid and pyridoxine deficiencies have not been noted as having any effect upon the eye.

SUMMARY

Both acute and chronic partial ariboflavinosis were produced experimentally in young and adult male and female C3H mice maintained on synthetic diets deficient in riboflavin.

The morphologic lesions demonstrated in both conditions were similar in genesis but more marked and more frequent in the chronic state. They consisted of (1) an atrophic and hyperkeratotic dermatosis followed occasionally by dermatitis, (2) myelin degeneration in the spinal cord, chiefly in the chronic-deficiency state, and (3) keratitis and infrequently a capsulolenticular type of cataract.

REFERENCES

(*1*) Sullivan, M., and Nicholls, J.: Nutritional dermatoses in the rat. IV. Riboflavin deficiency. J. Invest. Dermat., 4: 181–191 (1941).

(*2*) Unna, K.: Pantothenic acid requirement of the rat. J. Nutrition, 20: 565–576 (1940).

(*3*) Lippincott, S. W., and Morris, H. P.: Morphologic changes associated with pantothenic acid deficiency in the mouse. J. Nat. Cancer Inst., 2: 39–46 (1941).

(*4*) Sullivan, M., and Nicholls, J. M.: The nutritional approach to experimental dermatology. Nutritional dermatoses in the rat. I. Vitamin B_6 deficiency. J. Invest. Dermat., 3: 317–335 (1940).

(*5*) Foy, J. R., and Cerecedo, L. R.: Differences in the behavior of rats and mice towards deficiencies of certain members of the vitamin B complex. J. Nutrition, 22: 439–450 (1941).

(*6*) Engel, R. W., and Phillips, P. H.: Effect of riboflavin-low diets upon nerves, growth, and reproduction in the rat. Proc. Soc. Exper. Biol. & Med., 40: 597–598 (1939).

(*7*) Shaw, J. H., and Phillips P. H.: The pathology of riboflavin deficiency in the rat. J. Nutrition, 22: 345–358 (1941).

(*8*) Bethke, R. M., Record, P. R., and Kennard, D. C.: A type of nutritional leg paralysis affecting chicks. Poultry Sc., 10: 355–368 (1931).

(*9*) PHILLIPS, P. H., and ENGEL, R. W.: Neuromalacia associated with low riboflavin diets. A preliminary report. Poultry Sc., 17: 463–465 (1938).

(*10*) SEBRELL, W. H., and ONSTOTT, R. H.: Riboflavin deficiency in dogs. Pub. Health Rep., 53: 83–94 (1938).

(*11*) STREET, H. R., COWGILL, G. R., and ZIMMERMAN, H. M.: Further observations of riboflavin deficiency in the dog. J. Nutrition, 22: 7–24 (1941).

(*12*) PATEK, A. J., JR., POST, J., and VICTOR, J.: Riboflavin deficiency in the pig. Am. J. Physiol., 133: 47–55 (1941).

(*13*) LANGSTON, W. C., DAY, P. L., and COSGROVE, K. W.: Cataract in the albino mouse resulting from a deficiency of vitamin G (B_2). Arch. Ophth., 10: 508–514 (1933).

(*14*) BESSEY, O. A., and WOLBACH, M. D.: Vascularization of the cornea of the rat in riboflavin deficiency, with note on corneal vascularization in vitamin A deficiency. J. Exper. Med., 69: 1–12 (1939).

(*15*) KRUSE, H. D., SYDENSTRICKER, V. B., SEBRELL, W. H., and CLECKLEY, H. M.: Ocular manifestations of ariboflavinosis. Pub. Health Rep., 55: 157–169 (1940).

Effect of Foster Nursing on the Induction of Mammary and Testicular Tumors in Mice Injected with Stilbestrol

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The classic investigation of Loeb in 1919 and its subsequent confirmation and extension by Murray, Lacassagne, and others [1] established the fact that the estrogenic hormones are one of the essential factors involved in the genesis of mammary carcinoma in mice. Extensive work failed to reveal any correlation, however, between the susceptibility of various strains of mice to mammary tumors and the manifestations of estrogenic stimulation such as the duration, frequency, and normality of the estrus cycles. On the other hand, the study of the induction of mammary tumors in mice following administrations of estrogens demonstrated that there is a parallelism between the incidence of spontaneous mammary carcinoma in breeding females of various inbred strains and the readiness with which tumors of the breast can be induced by estrogens in male mice (*2*, *4*). This parallelism suggested that the estrogens were accelerators, in the female, and evocators, in the male, of some process already present or inherent in the animals.

The important studies on the extrachromosomal influences in the genesis of mammary cancer in mice culminated in the demonstration that at least one of the factors necessary for the occurrence of these tumors is an agent (or agents) transmitted through the milk from mother to offspring.[2] Foster nursing of mice of strains with a high incidence of mammary tumors by females with a low incidence of mammary cancer reduces markedly the incidence of tumors in the fostered females, and, conversely, foster nursing of mice of strains with a low incidence of mammary carcinoma by mice that develop such tumors increases the incidence of mammary cancer in the fostered females.

The relationship between the estrogenic hormones and the extrachromosomal factors in the appearance of mammary carcinoma was investigated by studying the effect of reciprocal crossing and of foster nursing on the induction of tumors of the breast with estrogens. Lacassagne (*6*) injected estrone into mice derived from reciprocal crosses between high-mammary-cancer strain RIII and low-mammary-cancer strains 17 and 39. Of 16 males and 15 females in which the maternal parent came from the high tumor strain, 13 males and 9 females developed tumors of the breast. Ten males and twelve females of which the maternal parent came from the low tumor strains did not develop mammary cancer. Twombly (*7*) implanted crystals of estrone into fostered and nonfostered male mice of strains C57 Black and RIII. Of 75 C57 Black males fostered by RIII females, 22 developed mammary carcinomas, whereas 35 C57 Black males nursed by their own mothers did not develop tumors. Mammary tumors arose in 29 of 59 RIII males nursed by their own mothers, and in 13 of 48. RIII

[1] *For reviews and bibliography, see Lacassagne* (1), *Gardner* (2), *and Loeb* (3).

[2] *For bibliography, see Bittner* (5).

males fostered by C57 Black females.[3] Bittner (*8*) implanted mice with pellets of estrone or estradiol benzoate. Of 13 females and 7 males of strain A that were nursed by A females (high tumor strain), 7 females and 3 males developed mammary tumors; 32 females and 16 males that were the offspring of strain A females nursed by C57 Black mice did not develop tumors. Three of five C3H females (high tumor strain) nursed by their own mothers developed cancer of the breast, whereas 8 similar females nursed by C57 Black mothers did not. Gardner (*9*), by means of weekly injections of estradiol benzoate, obtained 17 and 14 mammary tumors in 28 males and 24 females, respectively, that were derived by crossing CBA females (high tumor strain) with strain C57 males. Thirty females and twenty-three males of reciprocal crosses of these 2 strains did not develop mammary cancer.

Work on the effect of foster nursing on the induction of tumors in mice treated with estrogens was begun at the National Cancer Institute in February 1940. Since that time five separate experiments have been performed on male and female mice of three strains. A preliminary report of two experiments was published (*10*), and the present paper summarizes the final results. The development of testicular tumors of the interstitial-cell type in strain C mice (*11*) made it possible to observe also the effects of foster nursing on the induction of this neoplasm.

EXPERIMENTAL PROCEDURE

Mice of strains C3H, C, and C57 Black were used. The mice were born and raised at the National Cancer Institute. The derivation of the strains and the incidence of mammary and other tumors have been recorded by Andervont (*12*) for the C3H mice, by Shimkin, Grady, and Andervont (*11*) for the C mice, and by Little, Murray, and Cloudman (*13*) for the C57 Black.

For comparison with the experiments recorded here, table 1 presents the data on the incidence of mammary tumors in the breeding females of the three strains, and the effect of foster nursing on the occurrence of the neoplasm. When nursed by their own mothers, C3H mice are a high-mammary-tumor strain, whereas C and C57 Black mice are low-mammary-tumor strains. Strain C mice are susceptible to cancer of the breast upon the introduction of the milk factor by foster nursing, whereas strain C57 Black mice are relatively more resistant.

The mice used in the present investigation were kept under similar environmental conditions, with an unlimited supply of water, and upon a diet of Purina dog-chow pellets. Within 17 hours or less after birth, half of each C3H litter was transferred to a mother of strain C or C57 Black, and at the same time half of the litter of strains C or C57 Black was given to the C3H female. Litter mates were thus obtained, half of which were nursed by their own mothers and half were foster nursed by mothers of the other strains. The sexes and strains were separated at weaning and the animals marked indivi-

TABLE 1.—*Effect of foster nursing on the incidence of mammary carcinoma in breeding female mice*

Strain of mouse	Nursed by—	Number of mice	Mammary tumors		Average tumor age
			Number	*Percent*	*Months*
C3H	C3H	1,588	1,451	91.4	8.6
C3H	C57 Black or C	[1] 218	40	18.3	12.4
C	C	178	2	1.1	24–29
C	C3H	[2] 95	73	76.8	8.7
C57 Black	C57 Black	[3] 568	3	.5	15–24
C57 Black	C3H	93	28	30.1	15.2

[1] Includes only mice remaining with C3H mothers for 17 hours or less.
[2] Includes F_1 offspring of fostered C mice.
[3] Data from Little, Murray, and Cloudman (*13*).

[3] *Twombly, G. H.: Personal communication (1942).*

dually, so that fostered and nonfostered mice of each strain were kept in the same boxes and in close contact throughout the period of observation.

Stilbestrol [4] was used as the estrogen. The availability and the fact that the physiologic activity of this compound is apparently identical with the natural estrogens except for its high potency by the oral route made the use of stilbestrol highly satisfactory. The compound was either dissolved in sesame oil or used in the form of stilbestrol-cholesterol pellets. The preparation and the effects of the stilbestrol-cholesterol pellets were described previously (*11*).

The mice were examined weekly or semiweekly for the presence of external tumors and were killed as soon as the diagnosis of tumor was made in the gross. The subcutaneous and testicular tumors were sectioned and stained with haematoxylin and eosin for histologic verification. Mice that died or were killed before the appearance of tumors in the particular groups were not included in the tabulated results.

Experiment 1

Thirteen male mice of strain C3H nursed by C3H mothers and 22 male litter mates that were foster nursed by C57 Black females were injected subcutaneously once a week with 0.2 mg. of stilbestrol dissolved in 0.1 cc. of sesame oil. Twenty injections, for a total dose of 4.0 mg. of stilbestrol, were administered. The mice were 2 to 3 months old at the time of the first injection (Feb. 13, 1940). The experiment was terminated 15 months later, when the surviving mice were 17 to 18 months old.

Experiment 2

Male mice of strain C3H, nursed by their mothers or fostered by mice of strains C57 Black or C, and strain C males, nursed by their mothers or fostered by C3H females, received a single subcutaneous implant, into the right axilla, of a 25 percent stilbestrol-cholesterol pellet weighing 4 to 6 mg. A group of nonbreeding strain C females nursed by their mothers and the litter mates that were nursed by C3H females were implanted with similar pellets. Strain C57 Black males nursed by C57 Black or by C3H females were also used; about one-third of the animals received pellets containing 50 percent of stilbestrol, and the remainder received 25-percent pellets. The animals were 2 to 3 months of age at the beginning of the experiment (May 2, 1940).

Results, Experiments 1 and 2

Six tumors of the breast appeared after the publication of the preliminary report on these groups (*10*). The final findings on mammary tumors are given in table 2. The data on testicular tumors in the male C mice were included in another communication (*11*).

Mammary adenocarcinomas appeared in 3 of 31 C3H males that were fostered by C57 Black or C mice; of these 3 animals, 2 were with their own mothers for a maximum of 17 hours, and 1 was with its mother for 7 hours before transfer to a C female. Eighteen of twenty-two C3H mice nursed by their own mothers developed tumors. No tumors appeared in the C males nursed by their mothers, whereas the incidence of tumors in the C males fostered by C3H females was about 30 percent. In the C females, the incidence of 70 percent in the mice foster nursed by C3H females is in contrast with the development of 1 tumor in 34 mice nursed by their own mothers.

No mammary tumors were elicited in the C57 Black mice. This strain, however, tolerated the estrogen badly. The animals developed acute prostatitis within a

[4] *4,4'-dihydroxy-α,β-diethylstilbene, furnished by Merck & Co.*

TABLE 2.—*Effect of foster nursing on the induction of mammary carcinoma with stilbestrol in mice (experiments 1 and 2)*

Strain of mice	Nursed by—	Stilbestrol administered	Sex	Number of mice	Mammary tumors, in months after stilbestrol administration									Total tumors	Average latent time
					4	5	6	7	8	9	10	11	12	13		
															Number	*Mos.*
C3H	C3H	4.0 mg. in oil, 20 weekly injections.	M	13				1	3	1	1	1	2		9	9.4
C3H	C57 Black	do	M	22						1			1		2	10.5
C3H	C3H	25 percent stilbestrol-cholesterol pellet.	M	9			1	2	2		3	1			9	8.7
C3H	C57 Black or C	do	M	9									1		1	12.0
C	C	do	M	20											0	
C	C3H	do	M	13				1	2	1	1				5	8.4
C	C	do	F	34					1						1	8.0
C	C3H	do	F	20	1	6	2	1	2	2					14	6.2
C57 Black	C57 Black	25 or 50 percent stilbestrol-cholesterol pellet.	M	21											0	
C57 Black	C3H	do	M	17											0	

few days after the implantation of stilbestrol and later had extreme degrees of urinary retention or obstruction and scrotal herniation. Some of the surviving animals were killed at 11 months after the administration of stilbestrol, and several had markedly enlarged pituitary glands. Gardner (*9*) has shown that reciprocal crossing does not influence the development of pituitary adenomas in the hybrids of this strain.

EXPERIMENT 3

Fostered and nonfostered male mice of strains C3H, C, and C57 Black were implanted in the right axilla with single 10 percent stilbestrol - cholesterol pellets weighing 6 to 8 mg. The mice were 2 to 2.5 months of age at the beginning of the experiment (Sept. 9, 1940) and weighed 23 to 26 gm. The animals were weighed once a week for the first 4 months in order to determine whether any difference in this general response after the administration of stilbestrol could be elicited. The mice were examined weekly for the presence of subcutaneous growths, and the C males were checked for the incidence and latent time of the testicular tumors. The experiment was terminated 16 months after the implantation of the stilbestrol pellets.

Results

The data on the appearance of mammary carcinomas are presented in table 3, which includes the number of mice surviving without tumors as well as the number of tumors at each month.

The results are in agreement with the findings of experiments 1 and 2. Foster nursing of C3H males by C or C57 Black females reduced the incidence of mammary tumors from 46 to 16 percent. In the foster-nursed mice, 22 mice were with their own mothers for a maximum of 17 hours, 12 were with their mothers for up to 7 hours, and 9 were removed to the foster mother immediately after birth. In these groups, tumors developed in 4, 1, and 2 mice, respectively; both of the males that developed tumors (at 14 months) despite immediate removal from their mothers were fostered by C57 Black females.

Strain C males nursed by their own mothers did not develop tumors of the breast, whereas the incidence among their litter mates fostered by C3H females was about 30 percent. The high early mortality among the C males was due in part to the killing of mice that developed enlargement or frank tumors of the testis. Foster nursing did not exert an effect on the appearance of testicular tumors. These

data were combined with the findings of experiment 5 and are discussed under that section.

The results with C57 Blacks were inconclusive. Only one tumor appeared in the group fostered by C3H females. The mice tolerated the 10-percent pellets better than pellets with a higher concentration of stilbestrol, but the incidence of urinary retention and of herniation remained high.

The determinations of weight increment in these mice are recorded in figure 1. In this staggered graph, it is apparent that the weight gain was almost identical in mice of strains C57 Black and C, and that foster nursing exerted no influence on the weight increment. The weight data of strain C3H mice, although not influenced by foster nursing, were different from the data obtained with mice of strains C57 Black and C. The C3H mice gained 6 to 7 gm. during the total 4 months of observation, whereas the average gain for the other two strains was 4 to 5 gm. Moreover, whereas there was a definite retardation in weight increment in strains C and C57 Black mice during the period of 1 to 3 months following the implantation of the stilbestrol pellets, this effect was less apparent and occurred a month later (2 to 3 months) in the C3H animals.

It was found (*10*) that females of strain C3H required approximately twice the amount of estrone to produce a vaginal

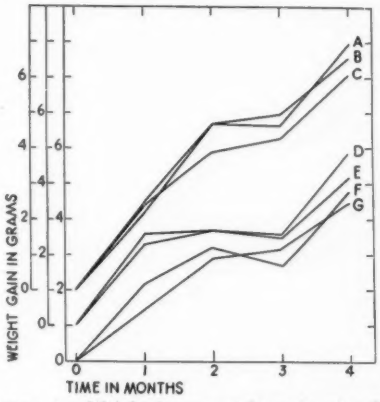

FIGURE 1.—Weight increment in male mice following implantation of 25 percent stilbestrol-cholesterol pellets subcutaneously. Mice were 2 to 2.5 months and weighed 23 to 26 gm. (experiment 3). A, C3H mice fostered by C57 Black females. B, C3H mice nursed by own mothers. C, C3H mice fostered by C females. D, C57 Black mice nursed by own mothers. E, C57 Black mice fostered by C3H females. F, C mice fostered by C3H females. G, C mice nursed by own mothers.

reaction than did females of strains C or C57 Black. The greater effect of stilbestrol on the weight increment in strains C and C57 Black males as contrasted with the effect on C3H males may represent another manifestation of the greater resistance of C3H mice to estrogens. The lack of effect of foster nursing again emphasizes the necessity of this procedure in demonstrating differences in mice which may be

TABLE 3.—*Effect of foster nursing on the induction of mammary carcinoma in male mice implanted with 10 percent stilbestrol-cholesterol pellets at 2 months of age (experiment 3)*

Strain of mouse	Nursed by—	Number of mice	Mice with tumors / Mice living without tumors in months after stilbestrol administration											Total tumors	Average latent time
			6	7	8	9	10	11	12	13	14	15	16		
														Number	*Months*
C3H	C3H	22	2/20	3/16	2/14	1/12	1/11	0/10	0/9	0/9	1/8	0/8	0/7	10	8.2
C3H	C57 Black or C	43	0/43	0/42	2/40	0/40	0/38	2/36	0/27	0/26	2/24	0/23	1/21	7	11.7
C	C	36	0/36	0/36	0/28	0/25	0/23	0/16	0/10	0/4	0/4	0/4	0/1	0	.....
C	C3H	28	0/28	1/27	2/22	2/19	0/15	1/14	2/11	0/9	0/5	0/5	0/2	8	9.5
C57 Black	C57 Black	13	0/13	0/13	0/13	0/13	0/13	0/13	0/11	0/10	0/8	0/8	0/6	0	.....
C57 Black	C3H	12	0/12	0/12	1/11	0/11	0/10	0/9	0/9	0/8	0/6	0/5	0/5	1	8.0

causally related to the genesis of mammary carcinoma rather than being incidental strain peculiarities.

Experiment 4

The female litter mates of the male mice used in experiment 3 were first set aside for a study of the effect of foster nursing on the vaginal response to estrone. The results of this investigation were reported previously (*10*). Fostered and nonfostered female mice of strains C3H, C, and C57 Black were ovariectomized at 6 to 8 weeks of age. For assays of the vaginal response, the mice during the next 16 weeks received subcutaneously 0.4 to 0.5 micrograms of estrone, divided into 11 biweekly injections at fortnightly intervals. Estrone was dissolved in purified peanut oil, and a total quantity of 0.6 cc. of the solvent was injected during this period into the right axilla or along the dorsal region.

At about 6 months of age (Jan. 7, 1941) the mice were implanted in the left axilla with single 25 percent stilbestrol-cholesterol pellets 4 to 6 mg. in weight. The experiment was terminated 12 months later, when the mice were about 18 months old.

Results

The results in the ovariectomized females, as given in table 4, are in agreement with the findings in the males and in the nonbreeding strain C females of experiment 2. The incidence of mammary tumors was radically reduced in strain C3H females fostered by C or C57 Black mice. Of the 32 fostered C3H mice, 13 were with their own mothers for a maximum of 17 hours after birth, 10 were with their mothers for no more than 7 hours, and 9 were removed to the foster mothers immediately after birth. The number of mammary tumors in these 3 groups was 3, 2, and 1, respectively. The mouse that developed a tumor despite its immediate removal from the C3H mother was fostered by a C female.

The incidence of mammary tumors was markedly increased in strain C females that were nursed by C3H foster mothers. No tumors appeared in strain C57 Black females of either group. In view of the results obtained by De Ome (*14*) in the breeding females of this strain, in which 21 percent of the mice developed tumors at an average age of 13.9 months, it is probable that the negative results of this experiment are best explained by the low survival of the animals.[5] The females developed pyometra and, like the males, rapid and extreme degrees of urinary retention.

[5] *The low incidence (approximately 10 percent) of mammary tumors in foster-nursed breeding C57 Black females previously reported from the National Cancer Institute* (15) *was also due to the early death of the mice. Recent experiments in which the survival was better corroborate the higher incidence obtained by De Ome* (14) *and are included in the results given in table 1.*

Table 4.—*Effect of foster nursing on the induction of mammary carcinoma in female mice* [1] (*experiment 4*)

Strain of mice	Nursed by—	Number of mice	Mice with tumors / Mice living without tumors in months after stilbestrol administration										Total tumors	Average latent time
			2	3	4	5	6	7	8	9	10	11	12		
														Number	*Months*
C3H	C3H	31	0/31	0/31	6/25	4/21	9/12	7/4	1/2	1/1	1/0	.....	.....	29	6.0
C3H	C57 Black or C	32	0/32	0/32	0/32	1/31	1/27	1/26	2/21	0/20	0/17	0/16	1/15	6	7.7
C	C	20	0/20	0/19	0/17	0/17	0/16	0/15	0/11	0/9	0/7	0/5	.....	0	.....
C	C3H	11	1/10	0/10	2/7	1/5	0/5	2/3	0/3	0/2	0/2	.....	.....	6	5.0
C57 Black	C57 Black	22	0/22	0/20	0/18	0/18	0/14	0/14	0/14	0/12	0/10	0/10	0/8	0	.....
C57 Black	C3H	24	0/24	0/21	0/21	0/20	0/20	0/16	0/14	0/12	0/5	0/3	0/1	0	.....

[1] Mice were ovariectomized at 2 months of age; 25 percent stilbestrol-cholesterol pellets were implanted when mice were 6 months of age.

Two female mice developed connective-tissue tumors. A rhabdomyosarcoma appeared in a fostered C female, and a fibrosarcoma was found in a C57 Black female nursed by its own mother. Both tumors arose in the right axilla at or near the deposits of peanut oil. The tumors appeared 9 to 10 months following the original administration of estrone in peanut oil, and it is believed that they are attributable to the solvent rather than to the estrogenic hormone. It was noted previously (*16*) and also in this experiment that peanut oil is tolerated badly in the subcutaneous tissue of mice, remaining unabsorbed and producing extensive tissue reaction for as long as 11 months after injection.

Previous studies (*10*) on this group of mice showed that strain C3H females required 0.08 micrograms of estrone for the elicitation of a vaginal reaction whereas the ED_{50} dose for ovariectomized mice of strains C and C57 Black was 0.04 micrograms, but that foster nursing exerted no influence upon the susceptibility of the mice to the hormone as tested by this response. The observations of the present experiment extend the conclusion that the susceptibility of mice to estrogen is not causally related to the appearance of mammary carcinoma.

Experiment 5

Strain C mice which were fostered by C3H females and which developed a 64-percent incidence of mammary tumors (*15*) were bred to their fostered brothers. This line of C mice is now in the F_6 generation, and the results on the breeding females of the F_1 and F_2 generations have been published (*17*). The male mice of the F_2 generation were implanted with 10 percent stilbestrol-cholesterol pellets weighing 6 to 8 mg., and the males of the F_2 generation received pellets of 25 percent stilbestrol-cholesterol weighing 4 to 6 mg. As controls, similar groups of C males nursed by nonfostered C females were implanted with 10 and with 25 percent stilbestrol-cholesterol pellets. The incidence and the time of appearance of testicular tumors were also recorded and combined with the results of experiment 3. The experiment was started when the mice were 1.5 to 3 months old and terminated 12 to 15 months later.

Results

The results on the appearance of mammary tumors are summarized in table 5. Mammary tumors were induced in the F_1 and F_2 male offsprings of C strain females that were nursed by C3H mice. The tumor incidence of 24 percent (6 tumors in 25 mice) in the F_2 generation compares favorably with the tumor incidence of 31 percent (13 tumors in 41 mice) obtained in strain C males fostered by C3H females in experiments 2 and 3. The average latent times, 8.8 and 9.1 months, respectively, are also in agreement. It is concluded that the milk factor obtained from the C3H females was transmitted through 2 generations of strain C mice after its original introduction into the line.

Table 6 records the incidence and latent period of testicular tumors that appeared in fostered and nonfostered C male mice of experiments 3 and 5. All the tumors were of the interstitial-cell type and were described in detail elsewhere (*10*). The latent-period observations presented some difficulty since all strain C mice following treatment with large doses of stilbestrol showed hyperplasia of the interstitial cells. The criteria of tumor were a hard nodular or smooth enlargement of the testis to twice or more normal size and its his-

TABLE 5.—*Induction of mammary carcinoma with stilbestrol-cholesterol pellets in male strain C mice, the offspring of strain C females foster nursed by C3H females (experiment 5)*

Description of mice	Stilbestrol concentration in pellet	Number of mice	Mice with tumors / Mice living without tumors in months after stilbestrol administration								Total tumors	Average latent time
			5	6	7	8	9	10	11	12		
	Percent										*Number*	*Months*
Sons of mice nursed by C3H females (F_1)	10	14	0/14	0/14	1/12	1/10	0/9	0/4	0/0		2	7.5
Mice nursed by C females	10	17	0/17	0/17	0/17	0/13	0/12	0/8	0/7	0/7	0	
Grandsons of mice nursed by C3H females (F_2)	25	25	1/24	2/23	1/20	1/15	1/12	0/10	0/6	0/6	6	8.8
Mice nursed by C females	25	21	0/21	0/20	0/20	0/13	0/13	0/11	0/8	0/1	0	

tologic appearance. It is shown in table 6 that the incidence and the latent period of primary interstitial-cell tumors of the testis induced with stilbestrol in strain C mice were not affected by the presence or absence of the milk factor in the mice.

DISCUSSION

Table 7 summarizes five experiments on the effect of foster nursing upon the incidence of mammary tumors in male and female mice of strains C3H, C, and C57 Black following massive treatment with stilbestrol.

The results with mice of strains C3H and C are clear cut. Foster nursing of C3H males and females by C57 Black or C mice reduces the incidence of mammary adenocarcinoma, and foster nursing of C males and females by C3H mice raises the incidence of tumors of the breast. The milk factor is transmitted in strain C mice to the F_1 and F_2 generations without apparent dilution. The results with strain C57 Black mice are indefinite, probably owing to the low survival of the mice after treatment with stilbestrol. It has been demonstrated by De Ome (*14*) and by Van Gulik and Korteweg (*18*) that the incidence of mammary tumors in breeding females of this strain can be increased to over 20 percent by foster nursing, and Twombly

TABLE 7.—*Summary of experiments on the effect of foster nursing upon the induction of mammary carcinoma in mice with stilbestrol.*

Strain of mice	Nursed by—	Sex	Number of mice	Mammary tumors		Average latent time
				Number	*Percent*	*Months*
C3H	C3H	M	44	28	63.6	8.7
C3H	C57 Black or C	M	74	10	13.5	11.5
C	C	M	94	0		
C	C3H [1]	M	80	21	26.2	8.8
C57 Black	C57 Black	M	34	0		
C57 Black	C3H	M	29	1	3.5	8.0
C3H	C3H	F	31	29	93.5	6.0
C3H	C57 Black or C	F	32	6	18.7	7.7
C	C	F	54	1	1.8	8.0
C	C3H	F	31	20	64.5	5.8
C57 Black	C57 Black	F	22	0		
C57 Black	C3H	F	24	0		

[1] Includes offspring of foster-nursed mice.

TABLE 6.—*Lack of effect of foster nursing upon induction of interstitial-cell tumors of the testis in strain C male mice implanted with 10 and 25 percent stilbestrol-cholesterol pellets (experiments 3 and 5)*

Strain of mice	Nursed by—	Number of mice	Testicular tumors in months after stilbestrol administration							Total tumors		Average latent time
			6	7	8	9	10	11	12			
										Number	*Percent*	*Months*
C	C	[1] 74	1	7	3	9	3	0	0	23	31.1	8.3
C	C3H	[2] 67	3	3	3	6	4	1	0	20	29.7	8.9

[1] Data from table 3, line 3, and table 5, lines 2 and 4.
[2] Data from table 3, line 4, and table 5, lines 1 and 3, thus including offspring of C females foster nursed by C3H mice.

(7) has reported similar results in males injected with estrogen. The C57 Black mice are more resistant to the influence of the milk factor than strain C mice, as shown by the lower incidence and the longer latent period of the tumors following similiar exposure to the milk influence.

Strain C3H mice are more resistant to the physiologic and toxic effects of stilbestrol and other estrogens than are mice of strains C or C57 Black. This is shown by the larger doses of estrone needed for C3H mice to produce the vaginal response, and the less marked effect of stilbestrol upon the weight increment of C3H mice (experiment 3) and upon the lower mortality following massive treatment with the hormone (experiments 2, 3, and 4). These differences are not causally related to the genesis of mammary tumors in the mice.

The presence or absence of the milk factor in strain C mice has no influence upon the development of primary interstitial-cell tumors of the testis following the administration of stilbestrol (table 6).

Mammary carcinoma in mice is the end result of a carcinogenic process acting on a suitable substrate. Before the demonstration of the milk factor, it was generally considered that the estrogenic hormones were the carcinogenic stimulus that determined the appearance of mammary tumors (*1–3*). In view of the present knowledge, however, it seems clear that both the genetic and the hormonal factors in the genesis of mammary tumors in mice should be relegated to the substratal role, while the factor or factors transmitted through the mother's or foster mother's milk [6] should be considered the chief stimulus of the carcinogenic process. All three factors, the genetic, the hormonal, and the MTI must be present for the development of mammary carcinoma. Thus, it has been demonstrated in the present report that unless the MTI is introduced into mice of strain C, genetic susceptibility of the animals and huge doses of estrogen do not lead to the development of mammary tumors. Also, male mice do not develop mammary carcinoma despite the presence of MTI unless the lacking hormonal factor is supplied. On the other hand, a quantitative deficiency of any one factor probably can be overcome by the relative quantitative increase in the other two factors. Thus, the relative genetic resistance to tumors in mice of strain C57 Black can be overcome by the presence of the MTI and sufficient estrogenic stimulation (*14–18*).

The genetic constitution of the mouse influences the degree of susceptibility of the animal to the development of mammary tumors. This susceptibility is transmitted through the paternal as well as the maternal parent (*20*). Little (*21*) has pointed out that at least in mice of strain Y, this susceptibility is associated with the yellow gene. Murray and Little (*22*) have shown that the extrachromosomal influence is 10 times more powerful than any possible chromosomal factor, and the concentration of the chromatin of the high-mammary-cancer strain does not return the cancer incidence to that obtained in the original cancer strain.

The estrogenic hormones are primarily concerned with the development of the mammary gland to a proper kind and degree of hypertrophy necessary for the manifestation of the carcinogenic process initiated by the MTI. Other hormones, such as those of the anterior pituitary and the adrenal, are probably also involved in the development of the mammary gland. The delicate quantitative balance between

[6] *Woolley, Law, and Little* (19) *recently designated the milk influence as the mammary tumor inciter, or the MTI. For convenience, this suggestion is followed hereinafter.*

the amount of hormonal stimulus and the incidence of mammary tumors, demonstrable either by the dose of estrogen administered to the male (*23*) or by the number of pregnancies in the female (*24*), indicates that the relationship between the hormonal stimulation and the carcinogenic process already present in the animal through the ingestion of the MTI is intimate and important.

With the proper genetic and mammary gland substrate, a mouse develops mammary carcinoma if it has received the MTI in the milk of its mother or foster mother. The nature of the MTI is unknown at present, although some of its properties are already apparent. Since the incidence of mammary tumors in mice is directly proportional to the time spent with the mother that will develop mammary cancer, rather than correlated to the time spent with the potentially noncancerous foster mother, it is apparent that the factor is carcinogenic rather than anticarcinogenic. It has been shown that the MTI is present in the milk of mice of the high tumor C3H strain throughout the period of lactation (*25*) and that it is probably widely distributed throughout the body, being present in the spleen, thymus, lactating gland, mammary tumor, and whole blood (*5*, *19*, *26*, *27*).

Despite its wide distribution, the action of the agent is quite specific, initiating as far as is known at present only tumors of the breast. It has been demonstrated that the MTI is not involved in the genesis of spontaneous pulmonary, hepatic (*12*), or lymphoid tumors (*28*), of pulmonary or subcutaneous tumors induced with carcinogenic hydrocarbons (29), or tumors of the testes, uterine cervix, or the pituitary induced with estrogens (*9*, *11*, *30*). Neither does the MTI alter the susceptibility of mice to estrone (*10*), to St. Louis encephalitis virus (*31*), nor to the development of morphologic alterations in the adrenals or ovaries of mice receiving large doses of estrogen (*11*). It has been indicated, however, that the presence of MTI in strain dba mice alters their susceptibility to the transplantation of some leukoses (*32*). The presence or absence of the MTI also produces morphologic differences in the architecture of the mammary gland (*9*, *11*, *18*); these changes perhaps represent the early precancerous alterations produced by the MTI.

The MTI is transmitted, under normal conditions, through the milk of the lactating mother or foster mother. It can also be transmitted by the subcutaneous injection of lactating gland, spleen, and whole blood (*19*, *26*, *27*) or by feeding of mammary tumor tissue (*33*). It is apparently not transmitted through bodily contact.

By relatively acute contact of the animal with the agent, such as through ingestion of milk for a few hours after birth or ingestion of milk from potentially cancerous mice at 1 month of age (*5*, *25*), a sufficient quantity of the MTI is transmitted to produce mammary tumors 8 to 15 months later. Moreover, the MTI is apparently transmitted through at least several generations of mice which under normal conditions have a low incidence of mammary cancer. The possible implication of this fact is that there is no apparent dilution of the agent; therefore, the agent is either self-reproducing or stimulates in each mouse into which it is introduced the production of a similar amount of the agent for transmission to the progeny. The recent work (*27*, *34*) on the concentration of the MTI in the milk and tumor extracts by ultracentrifugation is compatible with the view that the MTI may belong or be related to the viruses.

SUMMARY AND CONCLUSIONS

Foster nursing of mice of a strain with a high incidence of mammary carcinoma (C3H) by mice with a low incidence of this tumor (C57 Black or C) reduced markedly the incidence of mammary tumors in both males and females receiving large doses of estrogen. Foster nursing of mice of a strain with a low incidence of mammary carcinoma (C) by mice with a high incidence of this neoplasm (C3H) increased the incidence of tumors of the breast in male and female mice treated with estrogen.

Foster nursing does not affect the weight increment following stilbestrol administration, or the induction of testicular tumors of the interstitial-cell type with stilbestrol.

The estrogens prepare a suitable substrate in the breast of the mouse for the carcinogenic action of another agent, transmitted through the milk of the potentially cancerous mother or foster mother.

REFERENCES

(*1*) LACASSAGNE, A.: Les rapports entre les hormones sexuelles et la formation du cancer. Ergebn. d. Vitamin- u. Hormonforsch., 2: 259–296 (1939).

(*2*) GARDNER, W. U.: Estrogens in carcinogenesis. Arch. Path., 27: 138–170 (1939).

(*3*) LOEB, L.: The significance of hormones in the origin of cancer. J. Nat. Cancer Inst., 1: 169–195 (1940).

(*4*) SUNTZEFF, V., KIRTZ, M. M., BLUMENTHAL, H. T., and LOEB, L.: The incidence of mammary gland carcinoma and cancer age in mice injected with estrogen and in noninjected mice of different strains. Cancer Research, 1: 446–456 (1941).

(*5*) BITTNER, J. J.: Further studies on active milk influence in breast cancer production in mice. Proc. Soc. Exper. Biol. & Med., 45: 805–810 (1940).

(*6*) LACASSAGNE, A.: Confirmation, par des expériences de traitement à l'œstrone, du rôle prépondérant de la mère dans la transmission héréditaire du carcinome de la mamelle. Compt. rend. Soc. de biol., 132: 222–224 (1939)

(*7*) TWOMBLY, G. H.: Breast cancer produced in male mice of the C57 (black) strain of Little. Proc. Soc. Exper. Biol. & Med., 44: 617–618 (1940).

(*8*) BITTNER, J. J.: The influence of estrogens on the incidence of tumors in foster nursed mice. Cancer Research, 1: 290–292 (1941).

(*9*) GARDNER, W. U.: The effect of estrogen on the incidence of mammary and pituitary tumors in hybrid mice. Cancer Research, 1: 345–358 (1941).

(*10*) SHIMKIN, M. B., and ANDERVONT, H. B.: Effect of foster nursing on the response of mice to estrogens. J. Nat. Cancer Inst., 1: 599–605 (1941).

(*11*) SHIMKIN, M. B., GRADY, H. G., and ANDERVONT, H. B.: Induction of testicular tumors and other effects of stilbestrol-cholesterol pellets in strain C mice. J. Nat. Cancer Inst., 2: 65–80 (1941).

(*12*) ANDERVONT, H. B., with the technical assistance of MCELENEY, W. J.: Spontaneous tumors in a subline of strain C3H mice. J. Nat. Cancer Inst., 1: 737–744 (1941).

(*13*) LITTLE, C. C., MURRAY, W. S., and CLOUDMAN, A. M.: The genetics of non-epithelial tumor formation in mice. Am. J. Cancer, 36: 431–450 (1939).

(*14*) DE OME, K. B.: The incidence of mammary tumors among low-tumor strain C57 blk mice when foster-nursed by high-tumor strain A females. Am. J. Cancer, 40: 231–234 (1940).

(*15*) ANDERVONT, H. B.: The influence of foster nursing upon the incidence of spontaneous mammary cancer in resistant and susceptible mice. J. Nat. Cancer Inst., 1: 147–153 (1940).

(*16*) SHIMKIN, M. B., and GRADY, H. G.: Carcinogenic potency of stilbestrol and estrone in strain C3H mice. J. Nat. Cancer Inst., 1: 119–128 (1940).

(*17*) ANDERVONT, H. B.: Note on the transfer of the strain C3H milk influence through successive generations of strain C mice. J. Nat. Cancer Inst., 2: 307–308 (1941).

(*18*) VAN GULIK, P. J., and KORTEWEG, R.: The anatomy of the mammary gland in mice with regard to the degree of its disposition for cancer. Verhandel. d. k. Akad. v. Wetensch 43: 891–900 (1940).

(*19*) WOOLLEY, W., LAW, L. W., and LITTLE, C. C.: The occurrence in whole blood of material influencing the incidence of mammary carcinoma in mice. Cancer Research, 1: 955–956 (1941).

(*20*) ANDERVONT, H. B.: The influence of the paternal parent in determining the susceptibility of mice to spontaneous tumors. J. Nat. Cancer Inst., 2: 7–11 (1941).

(*21*) LITTLE, C. C.: The genetics of spontaneous tumor formation. *In* Snell, G. D., ed., Biology of the Laboratory Mouse, pp. 248–278. Blakiston Co., Philadelphia (1941).

(*22*) MURRAY, W. S., and LITTLE, C. C.: Chromosomal and extrachromosomal influence

in relation to the incidence of mammary tumors in mice. Am. J. Cancer, 37: 536–552 (1939).

(*23*) BURNS, E. L., and SCHENKEN, J. R.: Quantitative studies on relationship between estrogen and mammary gland carcinoma in strain C3H mice. Proc. Soc. Exper. Biol. & Med., 43: 608–610 (1940).

(*24*) JONES, E. E.: Tumor incidence in line A albino mice following injections of Progynon-B. Am. J. Cancer, 39: 94–99 (1940).

(*25*) ANDERVONT, H. B., and MCELENEY, W. J.: The influence of foster nursing upon the incidence of spontaneous breast cancer in strain C3H mice. Pub. Health Rep., 54: 1597–1603 (1939).

(*26*) BITTNER, J. J.: The influence of transplanted normal tissue on breast cancer ratios in mice. Pub. Health Rep., 54: 1827–1831 (1939).

(*27*) BRYAN, W. R., KAHLER, H., SHIMKIN, M. B., and ANDERVONT, H. B.: Extraction and ultracentrifugation of the mammary tumor inciter in mice. J. Nat. Cancer Inst., 2: 451–455 (1942).

(*28*) BARNES, W. A., and COLE, R. K.: The effect of nursing on the incidence of spontaneous leukemia and tumors in mice. Cancer Research, 1: 99–101 (1941).

(*29*) SHIMKIN, M. B., and ANDERVONT, H. B.: Factors influencing carcinogenesis with methylcholanthrene. II. Lack of effect of foster nursing. Pub. Health Rep., 54: 2080–2085 (1939).

(*30*) ALLEN, E., and GARDNER, W. U.: Cancer of the cervix of the uterus in hybrid mice following long-continued administration of estrogen. Cancer Research, 1: 359–366 (1941).

(*31*) WRIGHT, F. H.: Effect of foster-nursing upon inborn resistance of mice to St. Louis encephalitis. Proc. Soc. Exper. Biol. & Med., 45: 871–873 (1940).

(*32*) LAW, L. W.: Foster nursing and the growth of transplantable leukemias in mice. Cancer Research, 2: 108–115 (1942).

(*33*) BITTNER, J. J.: Preservation by freezing and drying in vacuo of milk-influence for development of breast cancer in mice. Science, 93: 527–528 (1941).

(*34*) VISSCHER, M. B., GREEN, R. G., and BITTNER, J. J.: Characterization of milk influence in spontaneous mammary carcinoma. Proc. Soc. Exper. Biol. & Med., 49: 94–96 (1942).

Absence of Hypervitaminosis D in Mice Subjected to Ultraviolet Radiation

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In the course of studies of tumor induction (*1*, *2*) large numbers of male strain A mice are being subjected to antirachitic ultraviolet radiation applied in repeated heavy doses over long periods of time. These animals must receive a certain amount of vitamin D as a result of the irradiation, in addition to that of the diet (Purina dog chow). Because of the possibility that this might be excessive in some cases, it has seemed pertinent to examine some of the animals for hypervitaminosis D. The findings, entirely negative, are recorded here.

Autopsies were performed on 15 mice, which had received various doses of ultraviolet radiation. All tissues except those of the nervous system were examined. The following tissues were fixed in Zenker acetic acid and stained with haematoxylin and eosin: lung, heart, aorta, thyroid and parathyroid glands, trachea, esophagus, forestomach, stomach, the small and the large intestine, pancreas, spleen, mesenteric lymph node, adrenal gland, liver, kidney, bladder, testicle, sternum, femur, and vertebra. In addition, pieces of heart, liver, kidney, stomach, and the small and the large intestine were fixed in 80 percent alcohol and stained by Von Kossa's method for calcium, and by the van Gieson technique.

Microscopic examination of sections from these organs showed no lesions of hypervitaminosis D. The criteria recognized as occurring in hypervitaminosis D and found absent in our mice were reported for the rat (*3*, *4*) and man (*5*) as consisting essentially of focal degeneration and calcification principally in the heart, aorta, kidney, liver, and stomach.

Table 1 gives a summary of the amounts of radiant energy to which these animals were subjected and an estimate of the vitamin D which may be presumed to have been formed in or on their skins. The method of exposure to, and the measurement of the incident radiation were described in a previous paper (*1*). The estimates of the quantity of vitamin D formed are based on values obtained by Knudson and Benford (*6*) for the energy required to form 1 unit of vitamin D in rat skin. These values were not measured for all lines of the mercury arc, and it was necessary to estimate the effectiveness of certain lines from values obtained by Bunker and Harris (*7*) and from the absorption spectrum of ergosterol.

In table 1 the estimates of vitamin D formed are for 1 cm.^{2}, which is the approximate area of the mice's ears exposed to the radiation. The ears, being almost free of hair, are those areas in which the skin is directly exposed. The total area of the animal exposed is about 25 cm.^{2}, and since it is possible that sterols on the surface of the hair may be changed into vitamin D and taken into the digestive tract in the course of grooming, the estimates might be somewhat higher. However, the estimates are made on the basis of vitamin D formation in the skin of rats and, at best, can give only a rough indication of the amounts formed in the course of irradiation of mice.

TABLE 1.—*Radiant energy to which strain A male mice were subjected and estimated amount of vitamin D formed*

Series	Source	Wavelength	Weekly [1] dosage ergs per square centimeter $\times 10^7$	USP units of vitamin D per square centimeter formed per week (estimated)	Time irradiated	Animals examined
				Number	*Weeks*	*Number*
BC [2]	Low pressure mercury arc	2537A	36.0	800	32	10
AC [2]	Intermediate pressure mercury arc	3130A and shorter	3.6	65	48	1
AG [2]			3.6	65	48	1
X [2]			9.9	160	47	1
AO [3]			4.3	70	42	3

[1] Only the 3130A and shorter wavelengths are taken into consideration, as these alone produce vitamin D.
[2] Radiation administered in equal doses 5 days per week.
[3] Radiation administered in a single dose 1 day per week.

Harris, Ross, and Bunker (*3*) found that daily oral administration of 50,000 units of vitamin D (in the form of irradiated ergosterol or tuna-liver oil) to rats produced histopathologic changes in kidney, heart, aorta, and lung. In the most heavily dosed series, BC (table 1), the estimated daily vitamin D formation (5 days per week) is 160 units per square centimeter. Assuming that this quantity of vitamin is formed over the whole area of the animal exposed (25 cm.^{2}), the total dose available to the animal would be 4,000 units, which is comparable with the dose that produces lesions in rats, if the differences in body weight of the animals is considered. This is a maximum estimate, and the absence of lesions in the mice suggests that it may be inordinately high.

In all the experiments cited, a high percentage of tumors of the ear developed as a result of the irradiation (*2*). Our studies show that within the range of dosage of ultraviolet radiation suitable for the study of tumor induction, hypervitaminosis D may be disregarded as a complicating factor. It is doubtful that such hypervitaminosis can be induced in mice by doses of radiation which the animals will tolerate.

REFERENCES

(*1*) BLUM, H. F., KIRBY-SMITH, J. S., and GRADY, H. G.: Quantitative induction of tumors in mice with ultraviolet radiation. J. Nat. Cancer Inst., 2: 259–268 (1941).

(*2*) GRADY, H. G., BLUM, H. F., and KIRBY-SMITH, J. S.: Pathology of tumors of the external ear in mice induced by ultraviolet radiation. J. Nat. Cancer Inst., 2: 269–276 (1941).

(*3*) HARRIS, R. S., ROSS, B. D., and BUNKER, J. W. N.: Histological study of hypervitaminosis D. Relative toxicity of vitamin D of irradiated ergosterol and tuna liver oil. Am. J. Digest. Dis., 6: 81–83 (1939).

(*4*) HAM, A. W.: Mechanism of calcification in the heart and aorta in hypervitaminosis D. Arch. Path., 14: 613–626 (1932).

(*5*) LIPPINCOTT, S. W.: Histopathological study of a fatal case of hypervitaminosis D. Am. J. Path., 16: 665 (1940).

(*6*) KNUDSON, A., and BENFORD, F.: Quantitative studies of the effectiveness of ultraviolet radiations of various wavelengths in rickets. J. Biol. Chem., 124: 287–299 (1938).

(*7*) BUNKER, J. W. M., and HARRIS, R. S.: Precise evaluation of ultraviolet therapy in experimental rickets. New England J. Med., 216: 165–169 (1937).

Anaphylaxis and Hemorrhage in Transplanted Tumors

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The influence of anaphylaxis upon transplanted normal tissues was investigated by Loeb (*1*) in 1917. Using guinea pigs sensitized to horse serum as the test animal and transplanted uterus or thyroid as the tissue, he observed that in general there was no effect, but that it may be possible that where "the second injection markedly affects the general state of health of the animal" the transplanted tissue may suffer.

In 1933 Apitz (*2*) tested the idea that anaphylaxis might produce hemorrhage in a transplanted tumor. He used two small groups of stock mice bearing the Ehrlich carcinoma. The first group was not sensitive, and neither group exhibited hemorrhage into the tumor. He concluded, however, that the experiments were not conclusive and that the question remained open. Apitz also observed the effect of injecting an antigen-antiserum mixture. The effect he observed was irregular and less than that produced by agar upon the same tumor. Others have studied the relationship of antigen-antibody mixtures to hemorrhage in tumors and to the Shwartzman phenomenon.

No attempt will be made here to correlate the results of the experiments reported in this paper with the large amount of work done with bacterial products. A review of that work up to 1937 is given by Shwartzman (*3*) in his monograph on local tissue reactivity. While great interest attaches to the possible interrelationships in these two fields the true relations are not known, and discussion of the matter at this time would be too speculative.

The experiments reported here are of an exploratory character and are reported at this time because they raise some interesting questions.

MATERIALS AND METHODS

The mice used were of inbred strains A, C, and C3H obtained from the Jackson Memorial Laboratories at Bar Harbor, Maine. Both sexes were used. The mice varied from 2 to 10 months in age. They were housed in groups of 6 to 10 in wooden boxes. The diet consisted of Purina dog chow and water ad libitum.

The serum used was normal horse serum prepared in the usual manner and sterilized by filtration. The serum was taken from three different batches, of which one was pooled serum from two horses. No chemical agents were added at any time. Before centrifugation the serum was stored in the frozen state. After centrifugation and filtration it was stored in glass at +4° C.

The tumor used was sarcoma 37 originally obtained from the Crocker Institute. In the laboratories of the National Cancer Institute it has been carried in strain dba mice exclusively for 8 years, but during these experiments it was carried in the subcutaneous tissue of mice of other strains for short periods. For transplantation healthy tumors were removed aseptically and carefully trimmed of normal tissue and necrotic tumor. A tumor pulp was then prepared and inoculated intracutaneously after the manner of Andervont (*4*).

The test animals were sensitized by an intraperitoneal injection of 0.5 cc. of

normal horse serum. Seven days later they were inoculated intracutaneously with the tumor. On the fourteenth day, when the tumors were 7 days old, the mice were again given 0.5 cc. of horse serum intraperitoneally. The control animals were inoculated with the same tumor pulp at the same time as the test animals and received the same final dose of serum. They differed from the test animals only in that they had not received the sensitizing dose.

Seven experiments were carried out with small groups of animals in which test and control animals were matched as to sex, age, and strain.

RESULTS

Sixty sensitized test animals were given the provocative injection. Of these 55 exhibited shock followed by hemorrhage and necrosis of the tumor. The first signs of shock were seen as early as 15 minutes after injection of the provocative dose, and the first hemorrhage in the tumor that was seen through the skin occurred within 1 hour. In 59 control mice no shock was observed, and there was 1 hemorrhage which involved only a small portion of the tumor.

The signs of shock observed in the mice in these experiments were erection of the hair, a tendency to irregular or labored respiration, and coldness of the animal to touch. The animals sat in a hunched position, or in severe shock lay completely flaccid, and moved reluctantly upon stimulation. Occasionally one died after about 1 hour, but nearly all the animals survived.

Hemorrhage into the tumor was observed through the skin as a purplish discoloration in the early stages. Later the overlying skin became necrotic, and by the following day the site of the tumor was marked by a dry black crust. The gross appearance of the tumors at this stage is shown in figure 1.

The early pathologic changes occurring in the tumors of the shocked animals were for the most part similar to those described by others (*2*, *3*, *4*, *5*) as following the administration of bacterial products and need not be described in detail here. In general the pathologic changes consisted of engorgement of the vessels, hemorrhage, and necrosis. The more superficial part of the tumor showed the first and most severe changes. After 18 to 24 hours the tumor and overlying skin consisted of a necrotic hemorrhagic mass surrounded by an inflammatory zone. However, there were usually some tumor cells which appeared viable at the periphery of the tumor. The condition of the bulk of the tumor cells under high magnification is shown in figure 2.

The effect of the reaction on tissues other than the tumor has not been studied in detail. There was, however, marked vascular engorgement of the stomach and upper intestine.

One experiment designed to test the antigen specificity of the reaction was carried out. In 10 mice that had been sensitized to horse serum, 0.5 cc. of rabbit serum given as a provocative injection on the fourteenth day did not produce shock or hemorrhage in the tumor.

Since both histamine (*6*) and peptone (*7*) are known to produce an anaphylactoid state, these substances were administered to animals bearing 7-day-old sarcoma 37. Other investigators (*2*, *8*) have reported the use of these substances in mice bearing transplanted tumors but have not reported hemorrhage in the tumors. In this work 10 mg. of histamine dihydrochloride dissolved in 0.5 cc. of saline was given intraperitoneally in a single dose. This represents 20 percent of a lethal dose

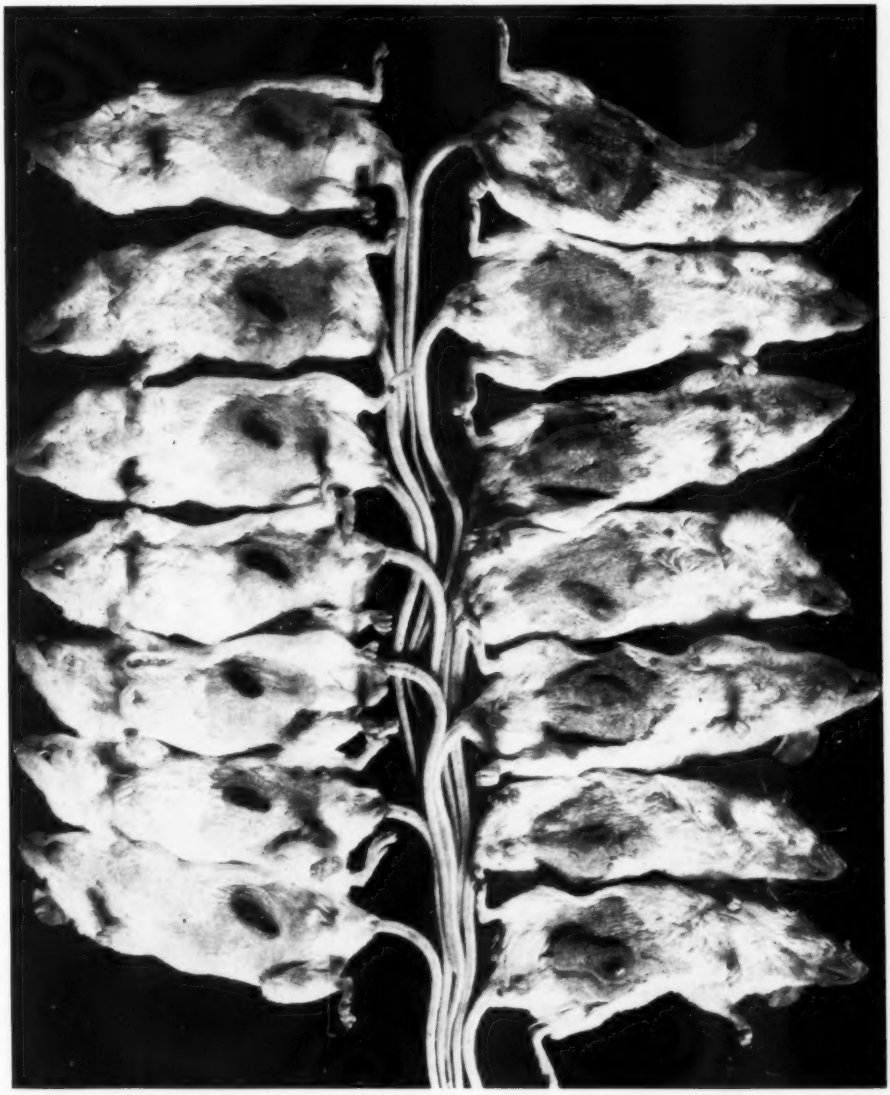

FIGURE 1.—Tumor S37 in strain C mice. All mice received 0.5 cc. of horse serum intraperitoneally 18 hours before this picture was made. The group on the left was sensitized. The group on the right is the control.

for these mice. It produced hemorrhage and necrosis in the tumor in 5 of 9 strain A mice. Twenty milligrams produced the reaction in all of 10 strain A mice. With peptone (Witte) the reaction was irregular and was obtained only with doses which

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were highly toxic. Negative results were obtained with a 20-mg. dose in 10 strain C mice. The same dose gave weak positive reactions in 5 of 10 strain A mice. Forty milligrams produced marked reactions in 8 of 10 strain A mice.

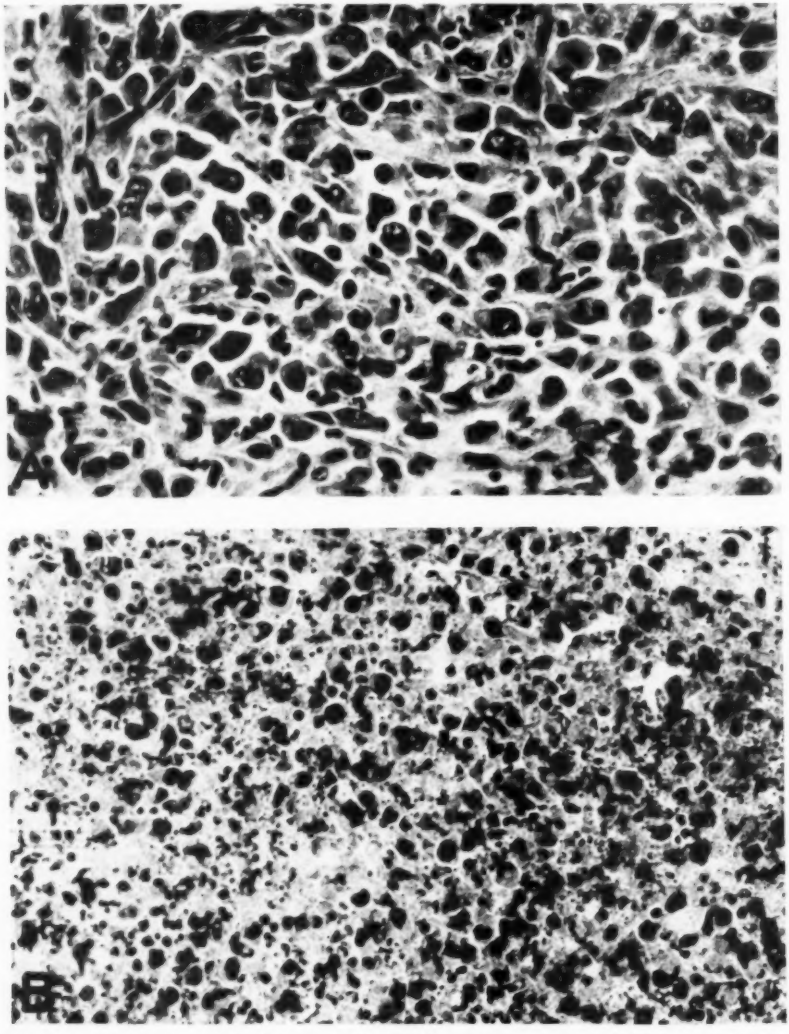

FIGURE 2.—*A*, tumor from a control mouse 18 hours after the provocative injection. × 425; *B*, tumor from a sensitive mouse at the same time. × 425. Both fields are taken from areas at a distance from the central core and are comparable. Zenker fixation, and haematoxylin and eosin stain.

DISCUSSION

It should be emphasized that these experiments are not presented as therapeutic. They represent an attempt to gain understanding of some of the principles which may influence induced hemorrhage and necrosis in tumors. The tumors are transplanted, and no inferences are drawn regarding spontaneous or primary induced tumors. For these reasons the percentage of ultimate regressions was not determined. Instead the animals were killed early for histologic study.

The optimal conditions of dosage, incubation period, strain of mouse, antigen, and type of tumor are not known at present, though all of these may have an influence upon the results obtained. These factors present some difficulty since it is well known that the mouse is a very refractory subject for the study of anaphylaxis and those animals that are better suited as subjects for anaphylaxis are not so good for the study of tumors. This phase of the problem is being attacked.

A few observations regarding favorable conditions have been made thus far: 0.5 cc. seems to be a better sensitizing dose than does 0.25 cc., and two sensitizing injections spaced 3 days apart seem better than one. The optimal incubation period is 12 days or longer. The phenomenon can be produced in strains A, C, and C3H, either male or female, and in both young and old adults.

With regard to the antigen, one would expect that almost any antigenic substance might be used but the mouse is difficult to sensitize and it may be very hard to hit upon optimal conditions. Horse serum has been used in the experiments reported here because it is easy to obtain and is a good antigen. It proved to be a fortunate choice. Shear (*8*) previously reported the use of horse serum and horse-serum concentrates in an attempt to influence the fluid balance of tumors. The serum was not used in such a way as to allow sensitivity to develop. His experiments were varied, and the reader is referred to the original article for details. In 1 group of 17 animals given 14 injections of 2 cc. each in a period of 19 days, he reported that the tumors "opened" in 10 of the animals and left "a hollow shell of tumor tissue." There was hemorrhage in 2 others. In other groups that received larger quantities these changes were not reported. Duran-Reynals (*9*) reported that rabbit, chicken, and horse serum when injected into tumor-bearing mice localized in the tumors. This might be of some importance in the present work though the relationship is not known. Crystalline egg albumin was used in 1 experiment with only moderate success. The mice were not strongly shocked, and mild hemorrhagic reactions occurred in only half of the animals. Other proteins have been tried in single experiments, but so far the mice have not become sensitive.

The reaction was not produced after an incubation period of 24 hours, and no local injection was given. This seems to separate it from the Shwartzman phenomenon though the two are probably related. It would seem that Forssman antigen can also be ruled out because both the mouse and the horse carry that antigen in their tissues.

One cannot fail to perceive similarities between the effects seen here and those produced by certain other biologic products. Just what the true relationships are is not known at present. A decision as to whether the phenomena are the same or only similar should be reserved for the future.

SUMMARY

In mice bearing 7-day-old cutaneous implants of sarcoma 37 the injection of normal horse serum was followed by signs

of shock and by hemorrhage and necrosis of the tumors if the mice had been previously sensitized to horse serum.

Similar doses of rabbit serum did not produce the reaction in animals sensitized to horse serum.

REFERENCES

(1) Loeb, L.: Tissue transplantation and anaphylaxis. J. Immunol., 2: 557–569 (1917).

(2) Apitz, K.: Über Blutungsreaktionen am Impfcarcinom der Maus. Ztschr. f. Krebsforsch., 40: 50–70 (1933).

(3) Shwartzman, G.: Phenomenon of local tissue reactivity. Paul B. Hoeber, Inc., New York (1937).

(4) Andervont, H. B.: The reaction of mice and of various mouse tumors to the injection of bacterial products. Am. J. Cancer, 27: 77–83 (1936).

(5) Gerber, I. E., and Bernheim, A. I.: Morphologic study of the reactivity of mouse sarcoma 180 to bacterial filtrates. Arch. Path., 26: 971–983 (1938).

(6) Dale, H. H., and Laidlaw, P. P.: The physiologic action of β-Iminazolyl-ethylamine. J. Physiol., 41: 318–344 (1910).

(7) Biedl, A., and Kraus, R.: Experimentelle Studien über Anaphylaxie. Wien. klin. Wchnschr., 22: 363–370 (1909).

(8) Shear, M. J. Studies on the chemical treatment of tumors. II. The effect of disturbances in fluid exchange on transplanted mouse tumors. Am. J. Cancer, 25: 66–88 (1935).

(9) Duran-Reynals, F.: Localization of foreign proteins and dyes in neoplastic growth. Proc. Soc. Exper. Biol. & Med., 36: 369–370 (1937).

Note on the Enzymatic Activity of a Transplanted Adenocarcinoma of the Glandular Stomach of a Mouse

By JESSE P. GREENSTEIN, *senior biochemist*, and HAROLD L. STEWART, *senior pathologist, National Cancer Institute, National Institute of Health, United States Public Health Service*

Stewart and Lorenz (*1*) have recently reported the induction of adenocarcinoma of the glandular stomach in mice. One of these tumors has been successfully transplanted in C3H mice (Bar Harbor strain) and is now in the ninth-generation transplant. It is called in this Institute mouse gastric adenocarcinoma No. 1. Since this tumor arises from that portion of the stomach where the enzyme-secreting cells are located, it is interesting to see whether the tumor retains the specific enzymatic activity of the tissue of its origin. It has already been demonstrated that the phosphatase activity of this tumor is markedly less than that in the normal mouse gastric mucosa, the acid phosphatase activity less by about a half, the alkaline phosphatase activity practically nonexistent (*2*). The present paper deals with the comparison of the pepsin and thymonucleodepolymerase activity of normal gastric mucosa and the transplanted adenocarcinoma of the pyloric stomach in mice.[1]

The tumors employed were the third-, fourth-, and eighth-generation transplants. Tumor transplants from six animals of each generation were used. The results obtained were substantially the same for all, the individual variations in enzymatic activity never exceeding 10 percent. Figure 1 represents the third-generation transplant of the tumor.

The tumors and the normal gastric mucosa were removed from the animals immediately after death and prepared for enzymatic determinations exactly as described earlier (*2*). Citrate buffers were used to stabilize the pH at the level desired. Two different protein preparations were employed as substrates for the

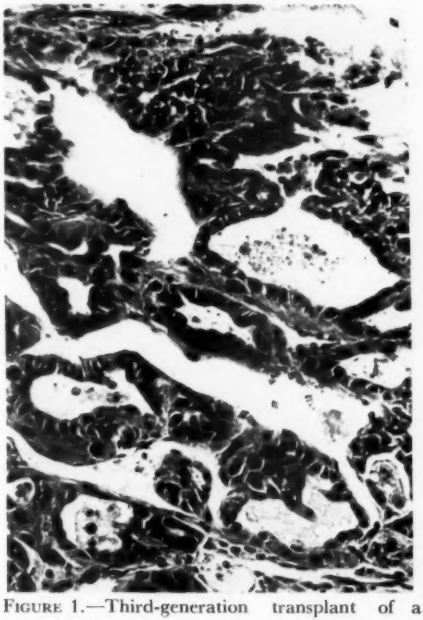

FIGURE 1.—Third-generation transplant of a methylcholanthrene-induced adenocarcinoma of the glandular stomach of a mouse. Photomicrograph. × 200.

[1] *The only investigation in the literature known to us of comparative enzymatic studies on an adenocarcinoma and on the normal tissue of origin is that of Sugiura, Pack, and Stewart* (3). *These investigators studied the amylolytic, lipolytic, and tryptic activities of an adenocarcinoma of the human pancreas and found that these activities were approximately the same as in normal human pancreas.*

TABLE 1.—*Proteolytic activity of normal gastric glandular mucosa and transplanted gastric adenocarcinoma*

[1.0 cc. substrate incubated at 37° C. with 2.0 cc. buffer and 0.5 cc. tissue extract]

Tissue	Concentration of N in extract	Time of incubation	Increase in tyrosine color value			
			Horse serum substrate		Egg albumin substrate	
			pH 1.39	pH 5.03	pH 1.39	pH 5.03
	Milligrams per cubic centimeter	*Minutes*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
Normal gastric mucosa in C3H mice	0.4	30	0.063	0	0.070	0
		120	.131	0	.136	0
Transplanted adenocarcinoma of the stomach in C3H mice	2.4	30	0	0	0	0
		120	0	0	0	0

determination of protease activity, egg albumin at 5-percent concentration, and horse serum at 1.6-percent protein concentration. For these determinations 1 cc. of the substrate plus 2 cc. of buffer was incubated at 37° C. with 0.5 cc. of the tissue extract, then treated with 5 cc. of the Folin phenol reagent (1:3), and centrifuged. Five cubic centimeters of the supernatant was removed and treated with 3 cc. of 20 percent sodium carbonate solution. The resulting colors were matched against appropriate tyrosine standards. The data on protease activity of the two tissues at two pH values are given in table 1.

Inspection of table 1 reveals the expected strong pepsin activity of the normal gastric mucosa at the low pH and the lack of this activity at the higher pH. No protease activity at either pH value, under the conditions employed, is apparent in mixtures containing extracts of the gastric adenocarcinoma.[2] A further check on these data was obtained by determinations of the milk-clotting activity of the two tissues. When 1 cc. of milk was treated with 0.1 cc. of $CaCl_2$ and 0.5 cc. of the tissue extracts at the concentrations given in table 1, the mixtures containing the normal mucosa extract clotted within 3 minutes, while the mixtures containing the tumor extract failed to clot even after 6 hours of standing.

Determinations of thymonucleodepolymerase activity were made as before (*4*, *5*). The substrate was preparation No. 2 of sodium thymonucleate (*6*). The method of calculation of activity has already been described (*5*). The data are given in table 2. For further comparison the results with small intestinal mucosa of strain A mice and transplanted adenocarcinoma originally induced in the small intestine of a strain A mouse (*5*, *7*) are included in table 2.

It is clear from table 2 that the thymonucleodepolymerase activity of the gastric

TABLE 2.—*Thymonucleodepolymerase activity of normal gastric and intestinal mucosa and of the transplanted adenocarcinomas of the stomach and intestine*

[3.0 cc. 1.0-percent sodium thymonucleate in water plus 3.0 cc. tissue extract, incubated in the viscometer at 30° C. Viscosity determinations made at external pressure of 16.0 cm. H_2O]

Tissue	Concentration of N in extract	Thymonucleodepolymerase activity
	Milligrams per cubic centimeter	
Normal gastric mucosa in C3H mice	1.23	6.05
	.41	1.82
Transplanted adenocarcinoma of the stomach in C3H mice	1.23	5.89
	.41	1.91
Normal intestinal mucosa in A mice (*5*)	1.23	14.74
	.41	8.49
Transplanted adenocarcinoma of the intestine in A mice (*5*)	1.23	14.41
	.41	8.52

[2] *Treatment of the tumor-digestion mixtures with cysteine failed to yield any noticeable protease activity at either high or low pH after 2 hours of digestion. When extracts of normal mouse liver were employed as the enzyme source, significant protease activity was observed with both kinds of substrates at pH 5.03.*

and of the intestinal tumors is practically the same as that of the respective normal tissues of origin. The activity of the gastric tissues is considerably less than that of the intestinal tissues.

The data so far obtained may be briefly summarized as follows: (1) The gastric adenocarcinoma possesses little, if any, peptic and alkaline phosphatase activity, whereas the normal tissue of origin, gastric mucosa, is rich in these enzymes; (2) the intestinal adenocarcinoma possesses little, if any, alkaline phosphatase activity, although the activity of this enzyme in normal intestinal mucosa is extremely high; and (3) the activity of thymonucleodepolymerase in the gastric and intestinal adenocarcinomas is the same as that in the respective normal tissues of origin. It would appear that when a normal tissue becomes neoplastic certain enzyme systems vanish or nearly vanish whereas other systems either remain the same or else even increase in activity. Studies of the activity of several enzyme systems in rat and mouse hepatomas and in normal liver emphasize this conclusion (*2, 4, 8–11*). Comparisons of normal and tumor tissues, based on enzyme studies, must hence be made not on one but on several enzyme systems.

REFERENCES

(*1*) STEWART, H. L., and LORENZ, E.: Induction of adenocarcinoma of the pyloric stomach in mice by methylcholanthrene (preliminary report). J. Nat. Cancer Inst., 2: 193–196 (1941).

(*2*) GREENSTEIN, J. P.: Distribution of acid and alkaline phosphatase in tumors, normal tissues, and the tissues of tumor-bearing rats and mice. J. Nat. Cancer Inst., 2: 511–524 (1942).

(*3*) SUGIURA, K., PACK, G. T., and STEWART, F. W.: A study of the enzyme content of a parenchymatous adenocarcinoma of pancreas and a comparison with the normal human pancreas. Am. J. Cancer, 26: 351–357 (1936).

(*4*) GREENSTEIN, J. P., and JENRETTE, W. V.: The depolymerization of thymonucleic acid by an enzyme system in normal and cancerous hepatic and mammary tissues and in the milk and sera of several species. J. Nat. Cancer Inst., 1: 845–863 (1941).

(*5*) GREENSTEIN, J. P.: A method of evaluating thymonucleodepolymerase activity in normal and tumor tissues. J. Nat. Cancer Inst., 2: 357–360 (1942).

(*6*) GREENSTEIN, J. P., with the assistance of JENRETTE, W. V.: Chemical studies on the components of normal and neoplastic tissues. I. Viscosity and streaming birefringence of sodium thymonucleate. J. Nat. Cancer Inst., 1: 77–90 (1940).

(*7*) LORENZ, E., and STEWART, H. L.: Intestinal carcinoma and other lesions in mice following oral administration of 1,2,5,6-dibenzanthracene and 20-methylcholanthrene. J. Nat. Cancer Inst., 1: 17–40 (1940).

(*8*) GREENSTEIN, J. P., JENRETTE, W. V., MIDER, G. B., and WHITE, J.: Chemical studies on the components of normal and neoplastic tissues. V. The relative arginase activity of certain tumors and normal control tissues. J. Nat. Cancer Inst., 1: 687–706 (1941).

(*9*) GREENSTEIN, J. P., JENRETTE, W. V., and WHITE, J.: The relative activity of xanthine dehydrogenase, catalase, and amylase in normal and cancerous hepatic tissues of the rat. J. Nat. Cancer Inst., 2: 17–22 (1941).

(*10*) GREENSTEIN, J. P., JENRETTE, W. V., MIDER, G. B., and ANDERVONT, H. B.: The relative enzymatic activity of certain mouse tumors and normal control tissues. J. Nat. Cancer Inst., 2: 293–299 (1941).

(*11*) MAVER, M. E., MIDER, G. B., JOHNSON, J. M., and THOMPSON, J. W.: The comparative proteinase and peptidase activities of rat hepatoma and normal and regenerating rat liver. J. Nat. Cancer Inst., 2: 277–282 (1941).

REVIEWS

Survey of Compounds Which Have Been Tested for Carcinogenic Activity. JONATHAN L. HARTWELL, associate chemist, National Cancer Institute, National Institute of Health, United States Public Health Service. 371 pp. (Multilithed) 1941. Available for free distribution, United States Public Health Service, Bethesda, Md.

Here collected for the first time in 1 volume are all the pertinent data on 696 chemical compounds tested in animals for carcinogenic potency up to the beginning of the year 1940. From the various papers reviewed, the important facts have been abstracted for the purpose of obtaining a comprehensive survey of a single limited field, that of chemical carcinogenesis. Included are some as yet unpublished material from the National Cancer Institute and much that has hitherto been virtually inaccessible in obscure foreign papers, which the author has consulted either in the original or in translation.

The volume is arranged in three parts. An introduction, together with a note on the scope of the work and chemical considerations, covers 11 pages. Under the heading "Chemical Considerations," the author discusses the nomenclature of the compounds commonly employed in the ever-enlarging field of experimental cancer research, which is of particular value to new investigators. The main body of the work is arranged in tabular form covering 284 pages in which the material is grouped according to chemical compound. Under each compound the pertinent data are listed in 10 categories: the reference, animal, strain, sex, preparation and dose, site and route, animals with tumors, survival, duration of experiment, and remarks.

Only single chemical compounds are considered. Such complex mixtures as the tars derived from a variety of materials, irradiated sterols, and articles of diet are excluded. Likewise mixtures of two or more pure compounds given simultaneously are not covered. Generally included, however, are crude grades of definite chemical individuals. The guiding principle has been to consider only such mixtures as render it feasible to determine to which constituent the given effects are attributed. Both inorganic and organic compounds are treated, and radioactive elements are also included since although their action may be generally due to physical radiation, in many cases the element is applied directly to the tissue and the possibility of chemical action cannot be rejected.

The author emphasizes the incompleteness of the data on most of the compounds listed, with the consequent limitation that must be imposed on deductions drawn from them, and enters a plea for the publication of more complete data in the future, doubly necessary in long-term work in which repetition by other laboratories is so costly. The most obvious needs are for more complete investigation of compounds already studied in a preliminary fashion and for filling large gaps in the list of new substances which it would seem desirable to test. As an example, the field of biologically occurring steroids is cited as hardly having been opened, although experiments of long duration have shown that certain members of this group, notably the estrogens, induce tumors locally at the site of injection, and in distant organs. Considering that these compounds occur in nature, an inquiry into the structural features responsible for their carcinogenic properties would appear to offer considerable promise of a greater understanding of the etiology of some types of cancer in man.

The author points out that in the United States there is an increasing amount of research being carried out on the numerous general problems depending upon chemical carcinogenesis, particularly the correlation

of carcinogenic activity with chemical structure. It is impressive that the 192 compounds which have been proven actively carcinogenic are found in a great variety of chemical classes and that substances of widely differing chemical structure possess similar carcinogenic activity; whereas, on the other hand, of two given chemical substances of the closest structural similarity one may, and the other may not be active. These considerations may at first thought make it seem hopeless to connect carcinogenicity in any way with chemical structure. However, it is the author's opinion that there is no justification for relaxing efforts in this direction of approach. Indications of what points should be revealed by studies along this line are the already-growing idea of the organ and tissue specificity and species selectivity among certain carcinogenic compounds. Further careful studies of previously inadequately tested compounds and compounds not yet employed may reasonably be expected to throw light on the etiology of different types of cancer.

The monograph is rendered easily usable by the inclusion of four indexes which cover the route and site of application, species of animal tested, site of tumor development, and vehicle in which the carcinogenic agent is carried. Finally, there are also a compound index and a bibliography.

HAROLD L. STEWART.

Cancer Mortality in the United States. MARY GOVER, associate statistician, U. S. Public Health Service, Division of Public Health Methods, National Institute of Health. Public Health Bulletins Nos. 248, 58 pp., 1939; 252, 74 pp., 1940; 257, 81 pp., 1940; and 275, 57 pp., 1941. For sale by the Superintendent of Documents, Government Printing Office, Washington, D. C., at 10 cents each.

The four bulletins listed above under the general heading "Cancer Mortality in the United States," constitute a study from the Division of Public Health Methods and the National Cancer Institute, of the National Institute of Health, in cooperation with the Division of Vital Statistics, United States Bureau of the Census. The subtitles of the bulletins give a more comprehensive idea of the subject matter: I. Trend of Recorded Cancer Mortality in the Death Registration States of 1900 from 1900 to 1935; II. Recorded Cancer Mortality in Geographic Section of the Death Registration States of 1920, from 1920 to 1935; III. Geographic Variation in Recorded Cancer Mortality for Detailed Sites, for an Average of the Years 1930–32; and IV. Age Variation in Mortality from Cancer of Specific Sites, 1930–32.

Each bulletin contains numerous tables and graphs, also an appendix made up of tables, thus presenting a statistical review based on the recorded deaths from cancer. A more detailed report of the subject matter, by bulletin, follows.

BULLETIN 248.—Cancer mortality for the death registration States of 1900 is given by specific age groups and by sex, for the period 1900 to 1935, for all cancer and for broad groups of sites. Age-site distribution has not hitherto been published by States. In certifying cancer as a cause of death there was an increasing tendency to include, on the death certificates, two or more organs as sites, which is one indication that more accurate diagnoses were made. Recorded mortality corrected to the age distribution of the population of the United States as of the 1930 census increased from 68.3 in 1900 to 120.6 in 1935 in the registration States of 1900. The increase was more rapid in the period 1900–1910 than it has been since 1910.

Cancer mortality.—Mortality by sex and age in each decade showed a greater rate of increase for males than for females. Among males there was an increase in rate at all age groups, this increase being most

marked above 65, and in this older age group the rate of increase remained constant from 1900 to 1935. Among females at younger ages (25–34), there has been no significant increase since 1910; at one age group (35–44), a decrease was noted since 1920. Over 65, the rate of increase has been less since 1910 than in the first decade.

Mortality for broad groups by site showed a marked increase, except for cancer of other or unspecified organs, during the period 1900–1910. Cancer of external sites, skin and buccal cavity, made no significant increase since 1910 for all ages combined, and the trends were similar for both sexes. Skin cancer decreased since 1920, at each age group, and for both sexes, except for females 85 and over. Cancer of the buccal cavity did not increase since 1920 at any age group and decreased at one (35–44). Among the specific sites included under the term "buccal cavity", cancers of the lip and of the tongue showed little increase since 1930, those of the mouth and other sites of the buccal cavity increased, and cancer of the jaw decreased. Presumably, modern methods of treatment of cancer at these sites may have been a factor in the mortality decrease with reference to these sites.

Mortality from cancer of the breast at all ages increased at a practically uniform rate after 1915; under 35 the increase was more rapid, while over 35 the increase was the same for each age group.

Cancer of the female genital organs, at all ages, showed a slight increase after 1910, no increase at 35 to 44, only a slight increase in the 24–34 and 45–54 age groups, and a definite increase over 55. Since 1930, the cancer rate for the more accessible sites, the uterus and other genital organs except the ovary, showed little change, and cancer of the ovary showed a marked increase. (It should be pointed out, however, that these and the foregoing data pertain only to years prior to 1935, the last date covered by this part of the study.)

Cancer of (1) the stomach and liver, (2) peritoneum, intestines, and rectum, and (3) other or unspecified organs, as a group, represent 91 and 55 percent for males and females, respectively, of the total rate for all cancer in 1930. For males, the larger increases were at the older ages, while for females the rates were downward from 1900 to 1930 at 35–44, level at the 25–34 and 45–54 age groups, and upward at over 55.

Among the internal organs more accessible of diagnosis, rates for the pharynx decreased, those for the esophagus, larynx, and bladder increased less than 2 percent, from 1925 to 1935.

For the stomach, liver, and biliary passages, and mesentery and peritoneum, the rates were level or slightly downward from 1925 to 1935. The decrease is probably accounted for by improved methods of diagnosis which resulted in a transfer of deaths from these to other internal sites.

Mortality from cancer of other internal sites, the pancreas, intestine, rectum and anus, prostate, kidney, lungs and pleura, brain, and bones, showed an annual increase of more than 2 percent, varying as high as 7 percent (for lungs and pleura). Again, improved methods of diagnosis are no doubt a factor in the increase in mortality. The rate of increase in cancer of the internal sites was higher for males than females.

BULLETIN 252.—The period covered in this part of the study is 1920–35, and this report deals primarily with the registration States of 1920. Rates have been adjusted to the age distribution of the total population in the census of 1930.

Cancer mortality.—The mortality is given

for five geographic sections of the United States, the Northeast, East North Central, South, West Central, and Pacific, and comparisons are made according to age, sex, State, and site of cancer. A comparison is also made of the cancer mortality of colored and white persons. The recorded mortality varied in different sections; the rate for females was higher than for males, and the order of the sections according to rate was the same for both sexes; the rate was highest in the Northeast (108.6 and 125.6 per 100,000 males and females), nearly the same in the Pacific and East North Central (97.2 and 95.5 per 100,000 males, and 114.9 and 113.6 per 100,000 females), somewhat lower in the West Central (82.5 and 100.7 per 100,000 males and females), and lowest in the South (60.6 and 91.2 per 100,000 males and females, respectively). For each of three 20-year age groups, mortality was also highest in the Northeast, nearly the same in the East North Central and Pacific, somewhat lower in the West Central, and lowest in the South. The relative variability from the average of the five geographic sections was more for males than females in the 35–54 and 55–74 age groups, but at 75 and over it was less than in the younger age groups and about the same for both sexes.

Rates for internal sites, (1) stomach and liver, (2) peritoneum, intestines, and rectum, and (3) other or unspecified organs, formed the major part of the mortality rates for males and slightly more than 50 percent of the total rate for all cancer for females. The mortality rate for the three groups in all sections was in the order given, save for the first group, the cancer of the stomach and liver among females in the Pacific section being comparatively low. The range of variability, in percentage of the average rate for all sections, was greatest for the second and least for the first group, for both sexes.

A relatively high rate was noted for cancer of the buccal cavity among females, and a high rate for cancer of the skin for both sexes in the South; the degree of variability in rates for cancer of the female genital organs was comparatively low; cancer of the buccal cavity (males) and of the breast (females) showed much the same sectional differences as those noted above for the internal sites.

Rates for sites common to both sexes were high for males, except the rate for cancers of the peritoneum, intestines, and rectum. In the South less relative difference was noted between rates for males and females for given sites. Mortality increased in all sections more rapidly for males than females; the rate of increase was practically the same for both sexes in the Northeast, East North Central, West Central, and Pacific sections, and slightly higher in the South.

Some specific trends were noted: a decrease in cancer of the buccal cavity in the Northeast and East North Central, an increase in the West Central and South, and a level rate in the Pacific section; a decline in the mortality curve for cancer of the skin (both sexes) and at practically the same rate in the Northeast, East North Central, and West Central, and a slight decline for females in the South; increase in cancer of the breast; the same sectional differences for cancer of the female genital organs as for cancer of the breast; a decline in mortality from cancer of the stomach and liver, greater for females than males, at practically the same rate for both sexes, in all sections except the South, where the rate for males remained level since about 1925; increase in cancer of the peritoneum, intestines, and rectum, generally higher among males; increase in cancer of other

or unspecified organs at approximately the same rate, and higher among males than females.

Age specific rates by section and site of cancer varied more than did the rate for all ages so that differences in trend are not usually significant. Cancer of the buccal cavity (males) decreased most in the youngest age group, 35–54, and either decreased or remained constant in all sections for this group; increase for 75 and over was less in the Northeast and East North Central than elsewhere; cancer of the skin among males in the 55–74 age group declined; increase in the breast-cancer rate did not vary much with age; in the 35–54 age group the increase in cancer of the female genital organs was slight, but the rate increased with advancing age in each section; cancer of the internal organs increased with age.

Mortality from cancer was lower for colored than for white males and lower in the South than in the North, but higher for colored than for white females and also lower in the South. The ratio of colored to white male was 84 and 67 percent, and for colored to white female 112 and 111 percent, in the North and South, respectively. Rates for all cancer in 20-year age groups was higher for colored than for white in the younger, but lower in the older age groups. For the three groups of internal sites previously mentioned, mortality was consistently lower among colored than white persons and lower in the South than in the North. Among the external sites certain differences were noted, as, for instance, the relatively high rate for cancer of the buccal cavity among colored females and the low rate of skin cancer for both sexes among the colored.

BULLETIN 257.—This report deals with average cancer mortality as recorded in the 3 years 1930–32 for all States with the exception of Texas. All rates are corrected to the standard age distribution of the total population as enumerated in 1930. The rates are specific for sex, and in States where the colored population is relatively large, for color also.

Cancer mortality.—The rates varied greatly in the different States, with a range of variation from 127 in Rhode Island to 56 per 100,000 in Arkansas. While the rates for all specific sites of cancer in a given section tended to be higher or lower than the average, there were some exceptions: the rates in the South were high for skin cancer (both sexes), for all sites of the buccal cavity (females), cancer of the pharnyx and mouth (males); cancer of the breast was low in the South and cancer of the uterus about average but relatively higher than for most of the other forms of cancer in the South; rates for cancer of the uterus were low in Wisconsin, Minnesota, Iowa, North Dakota, and South Dakota; rates for cancer of the stomach and of the whole digestive tract were high in all sections except the West South Central and the South; cancer of the respiratory organs was relatively high in the Northeast and the Pacific sections.

Cancer mortality in the colored population, represented almost entirely by the Negroes in the southern area, was compared with that of whites in the same area and with that of the colored in the Northern States. While the mortality for colored was generally lower than for white persons, the rates for some specific sites were higher in the former. Among colored males, rates for cancer of the scrotum in the South, and of the scrotum, tongue, stomach and duodenum, prostate, and mesentery and peritoneum in the North were higher than the corresponding rates for white males; among colored females, cancer of the uterus, jaw, pharnyx, rectum

and anus, stomach and duodenum, and bladder in the North were as high as or higher than the corresponding rates for white females. The recorded rate for all forms of cancer among colored males was slightly more than twice as high in the North as in the South, and among colored females one and one-half times as high in the North as in the South. Cancer of the skin in colored persons was nearly the same in the North and South.

The resident mortality rate for all cancer was highest in large cities and lowest in rural areas. The percentage of the population of States living in cities of 10,000 or more was used as an index of the amount of urbanization and was related to the rate for specific sites of cancer in each State. Mortality rates were generally higher in States having a large percentage of urban population.

When the percentage of deaths from cancer occurring in hospitals was held constant, and cancer mortality was correlated with the percentage of the population in cities of 10,000 or more, a large significant positive correlation was found for all cancer and for each of four sites, indicating an association between cancer mortality and urbanization apart from the hospitalization factor. A slight association was also noted between cancer mortality and population when urbanization was held constant. A comparison of correlation coefficients indicated that urbanization or factors related thereto were more closely correlated with the death rates of uterine cancer than were birth rates.

BULLETIN 275.—This report deals with the same period and the same States as does Bulletin 257. Variations in age curves of mortality from specific sites were compared for males and females, for white and colored, and for different geographic sections. The relative age curves were distinctive for specific sites. Cancer of the skin, for instance, was found to increase at a moderate and almost constant rate through life, cancer of the uterus to increase rapidly in women under 30 and to remain almost level after 65, and cancer of the brain to increase slowly up to 55 and then to decrease. Cancers of the brain, bones, respiratory and genital organs have a mean age at death under 60, those of the digestive tract and buccal cavity between 60 and 70, and of the skin and prostate at over 70.

Cancer mortality.—The most frequent sites for different age groups were found to be as follows: Under 5, kidney; 5–14, bones and brain; 26–64, uterus and breast among women; for men 35 and upward, and for women 65 and upward, stomach; for persons over 75, cancer of the prostate second and of the skin sixth for both men and women.

Among the external sites common to both sexes, buccal cavity, pharynx, esophagus, and larynx were relatively higher for men than women than were the internal sites, and the rate for men increased more rapidly at approximately 40 and 50. Deaths for men showed a greater concentration at 60–70, and the decline in the rate of increase of rates over 70 was greater The mean age at death for sites common to both sexes was practically the same, but the standard deviations of the age distributions were generally less for men.

Under age 55, rates for the digestive tract and genital sites were higher for colored than for white, but the same or lower for other sites. Over 55, the rates in the South increased less rapidly for

colored than for white, except in the case of cancer of the male genital organs excluding the prostate, and of the uterus.

A difference was noted in mortality at specific ages for specific sites in the different geographic sections, which consisted in a greater variability of section rates at 45–64 than at older ages. However, the sectional differences for specific sites were minor. The uniformity of the curves for specific organs in different sections, on the other hand, was striking

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